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REVIEW ARTICLE

Steroid Hormone Receptors and Endocrine Management of Mammary Carcinoma

Three recent developments have profoundly altered the practice of endocrine management of advanced mammary carcinoma Firstly the development of combination chemotherapy has offered treatment superior to endocrine therapy in unselected groups of patients Secondly the demonstration of steroid hormone protein receptors in mammary carcinomas sensitive to endocrine therapy has shed light on the mechanism of action of endocrine therapy and allowed selection of patients with hormone-dependent tumours Thirdly the development of antiestrogenic drugs has offered a possibility of endocrine treatment without the side effects of additive endocrine therapy This review will deal with possibilities and limitations of steroid hormone binding studies and of antiestrogen therapy of mammary carcinoma

After the demonstration of Folca et al in 1961 that administered ^3H hexestrol was accumulated in the tumours of four patients who responded to adrenalectomy a search was made for the presence of estrogen receptors in mammary carcinoma In a pioneering study Jensen et al (1) were able in 1971 to subdivide mammary carcinomas into estrogen receptor positive and estrogen receptor negative The latter were found to be resistant to adrenalectomy These data were rapidly extended and at a conference in Washington in 1974 (3) several groups reported that estrogen receptor negative tumours demonstrated a low probability of response to adrenalectomy or oophorectomy or additive therapy with estrogens or androgens In contrast estrogen positive tumours had an approximately 50% probability of response to additive or ablative endocrine therapy The reason why only about half of the receptor positive tumours responded remained to be explored This question was approached by several groups who applied refined quantitative assays for estradiol binding to mammary carcinomas and correlated the data to the results of therapy Several methods for the quantitative measurement of estrogen receptors in mammary carcinomas became available and the most important are described below All these depend on obtaining a fresh surgical or needle biopsy which is either immediately frozen at −70 C or kept cold and analysed within approximately two hours The biopsy is homogenized either before or after incubation with tritiated estrogen (usually 1–5 nM estradiol) The amount of radioactivity bound to the specific receptor is accepted as a measure of the amount of receptor in the tumours Three commonly used methods both physically characterize and separate the receptor protein from unbound steroid These are sucrose gradient centrifugation agar gel electrophoresis and the recently developed isoelectric focusing in polyacrylamide gel (2 5) Two other common methods are based on the fact that unbound steroid can be removed after absorption to dextran coated charcoal or that specific receptor can be removed from the tumour homogenate by the binding to protamine sulfate (2)

Several sources of error complicate estradiol binding assays Estradiol binds unspecifically to albumin and specifically to the transport protein sex hormone binding globulin (SHBG) Both proteins frequently contaminate tumours Both albumin and SHBG cosediment in sucrose gradients with the 4S form of the estradiol receptor The unspecific binding to albumin has a high capacity and its unspecific nature is often demonstrated in control incubations with excess of non radioactive steroid Agar gel electrophoresis and electrofocusing separate the estradiol receptor from albumin and SHBG and avoid these sources of error I prefer the latter method it isolates the receptor as a very sharp peak at pH 6 6 and this isolation is so specific that the receptor can be measured truly saturated after incubation with a large excess of radioactive steroid With the commonly used method based on dextran coated charcoal the specificity of binding is often determined after incubation of the tumour cytosol with different concentrations of radioactive steroid and estimation of binding constant by the graphical method of Scatchard (2)

The amount of estradiol receptor demonstrated

with one of these methods is then correlated to the amount of protein the amount of DNA or the wet weight of the tumour specimens Recent data using the most sensitive techniques e g isoelectric focusing demonstrate that approximately 85% of primary or metastatic breast cancers contain at least some estradiol receptor The amount of receptor contained varies within the range of three orders of magnitude from about 5–2000×10^{-15} moles (f moles) per mg of tumour tissue protein or 0 01–10 *f mole per μg of tumour DNA When this broad* range became clear it was realized that the subdivision of mammary carcinomas into estrogen receptor positive and estrogen receptor negative was a major oversimplification Recent studies have in fact demonstrated a correlation between the amount of receptor in a mammary carcinoma and an increased probability of response to endocrine therapy We do not understand however why some mammary carcinomas which contain large amounts of receptor do not respond to endocrine therapy

Two clinically important factors greatly influence the probability of demonstrating significant amounts of estradiol receptor The first is the patient s age the second is the tumour histology The older the patient the greater the probability of finding a high receptor value We have approached the first factor by measuring both the receptor content in the patient s tumour and the amount of circulat estradiol in the patient on the day of operation with Drs Theve and Carlström at Sab- g Hospital our group has demonstrated that ... with more than approximately 150 pM estradiol in the plasma have "receptor negative" tumours This most probably is an artifact dependent *on in vivo binding of the receptor by endogenous estradiol* I therefore think that receptor values obtained in menstruating women must be interpreted with caution and if data are considered necessary the women probably should be operated during the first third of the menstrual cycle—when the endogenous estradiol levels are low—in order to get reliable specimens for receptor analysis

It has long been known that decreasing the estradiol level with oophorectomy or adrenalectomy or increasing it in elderly women by additive therapy with a potent estrogen may result in tumour remissions It is still incompletely understood why decreasing and increasing serum estradiol may both result in tumour remissions We have however investigated the cytokinetic mechanism behind tumour remissions during these two types of endocrine therapy The common mechanism is a decreased fraction of cells in the S phase registered 1–3 weeks after therapy and a continued low level of cell replication until tumour remission is evident (4) Thus there appears to be no immediate tumour cell death but rather—after the stop of cell replication—a shift of the equilibrium between cell renewal and cell decay resulting in a slowly diminishing tumour

Concerning the second clinically important factor the correlation between estradiol binding and tumour histology the data are still conflicting Several groups are however approaching this problem which may be clarified within the next few years

When the synthetic antiestrogens became available their effects on mammary carcinomas were evaluated Around 1974 it was evident that therapy with an antiestrogen such as Tamoxifen resulted in tumour remission in approximately half of the elderly women treated (2) This fraction is very close to that observed with additive therapy with potent estrogens Compared to the side-effects observed with pharmacological amounts of active estrogens those observed with antiestrogens are very mild Due to this very large difference in side-effects additive estrogen therapy was rapidly abandoned in most centers and replaced by antiestrogen therapy It was considered so important to avoid the side effects of additive estrogen therapy that the change to antiestrogen therapy was generally performed without prior comparative studies in which the new therapy was evaluated in patients resistant to estrogen Therefore we do not know whether the group *of patients responding to antiestrogen is exactly the* same as that which used to respond to estrogen therapy There is however reason to believe that these two forms of therapy can help approximately the same group of patients (See below)

Several teams have tried to correlate the results of antiestrogen therapy to the estradiol binding observed in the tumours treated Our initial data demonstrate a very strong correlation between estradiol binding and the response of mammary carcinomas to antiestrogen therapy (5) Our first series of patients indicates that most patients with more than 0 2 f moles of receptor per μg of DNA respond to antiestrogen whereas only a very few with low amounts of receptor or no receptor respond to therapy Since it was previously demonstrated that

there is a positive correlation between the response to additive estrogen therapy and the amount of receptor (2 3) the two observations must mean that approximately the same group of patients responds to both estrogen and antiestrogen therapy We do however need more information on the quantitative receptor values associated with tumour remissions and we also need to correlate the duration of remission with the amount of receptor present in the tumours

What might future patients expect from the ongoing research on steroid hormone receptors and antiestrogens? Firstly I think it is reasonable to assume that better quantitation of the estrogen receptor will allow better predictions of results of therapy These better data may in part be derived from improvements in present techniques but more important progress must depend on the purification of the estradiol receptor the development of antisera to the receptor and then the establishment of radioimmunoassays and/or immunofluorescence assays These would enable measurement of receptors occupied by endogenous hormone and thereby give better information on the nuclear and cytoplasmic receptors in tumours from menstruating women A second major improvement may be due to present experiments in which estradiol binding androgen binding progesteron binding and glucocorticoid binding are all measured in the same mammary carcinomas and this profile of hormone binding is correlated to results of endocrine therapy These profile studies may enable more accurate predictions than those which can be obtained with estradiol binding only

A third and perhaps more important possibility is being investigated in several prospective clinical trials These are concerned with whether prophylactic antiestrogen therapy after mastectomy may prolong the patient s life They are also exploring the possibility of a correlation between the amount of steroid hormone binding of the primary carcinoma and the results of prophylactic antiestrogen therapy Hopefully we will some years from now be convinced that if we recommend postoperative antiestrogen therapy to a patient with an estradiol binding tumour her life will be prolonged We already know that we can give her the medication without troublesome side effects

Bo Nordenskjold Radiumhemmet Karolinska Hospital Stockholm Sweden

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Obstructive Characteristics of Bjork–Shiley, Hancock, and Lillehei–Kaster Prosthetic Mitral Valves in the Immediate Postoperative Period

Jarle Holen Johan Høie and Bjarne Semb

From the Departments of Radiology and Surgery A and the Institute for Surgical Research University Hospital Rikshospitalet Oslo Norway

ABSTRACT The flow obstruction in mitral valve implants was studied in the immediate postoperative period in 19 patients with Björk–Shiley, Hancock, or Lillehei–Kaster prosthetic mitral valves The effective valve area (A_e) was used as a measure of the flow obstruction The blood velocity in the implants was estimated with a non invasive ultrasound Doppler system The cardiac output was determined with an indwelling thermodilution catheter The collected data allowed the determination of A_e Multiple determinations of A_e at various cardiac outputs and pulse rates were generally performed in each patient during the first 2–3 postoperative days The investigation demonstrated that the method employed was useful for the study of mitral implant performance The obtained values of A_e demonstrated that the flow obstruction in prosthetic mitral valve implants is frequently considerable

A variety of prosthetic mitral valves have been introduced in recent years The valves have generally been tested in mechanical devices and animals but the ultimate test of a prosthetic valve s quality is its performance as an implant in a patient One of the chief parameters of interest in this respect is the flow obstruction in the implanted valve This obstruction is usually quantified from the pressure gradient and flow rate determined during cardiac catheterization A recent report (5) describes a method whereby the obstruction is quantified from non invasive ultrasound data and the cardiac output using the effective valve area (A_e) as a measure of the obstruction

In this hospital an indwelling catheter system has been found useful and is frequently employed in the immediate postoperative period to monitor cardiac output and other hemodynamic parameters in patients who have undergone mitral valve replacement In this situation it should be possible to use the ultrasound method to study the flow obstruction in mitral valve implants by performing multiple determinations of A_e in a patient during the first few postoperative days

In the present investigation the aforementioned ideas have been applied to the study of the flow obstruction in Bjork–Shiley Hancock and Lillehei–Kaster prosthetic mitral valves

PATIENTS

Data were collected from 19 adult patients with prosthetic mitral valves during the first 2–3 postoperative days The selection of valve type to be received by any given patient and the selection of patients for the present investigation were independent of the patient The prosthetic valves were implanted through a midline incision Bjork–Shiley and Lillehei–Kaster valves were positioned so that the larger opening was situated posteriorly The indwelling thermodilution catheter was usually inserted on the operating table Initial data collection was generally attempted within 24 hours after surgery at which time the patient was usually on a respirator and the heart rate was controlled by an external pacemaker Further data on the patients are presented in Table I

METHODS

Cardiac output was determined with the thermodilution principle Ten cm³ of 5% glucose solution at 0°C were rapidly injected by hand into the superior vena cava via the indwelling catheter (Swan-Ganz) while a temperature sensor registered the time course of the temperature in a pulmonary artery The cardiac output was computed electronically and presented as a digital display Prior to each determination appropriate checks were performed to ensure proper placement of the catheter

Ultrasound system A modified (3) 2 1 MHz non directional continuous waveform Hewlett Packard 8026B Sound Monitor was used to obtain the frequency shifts due to the blood velocities in the mitral valve im

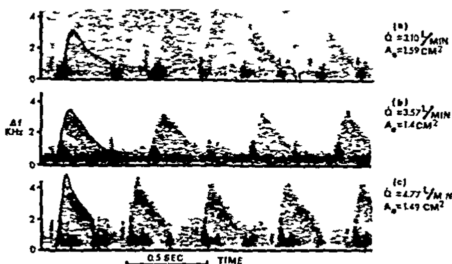


Fig 3 Patient 15 (Hancock 79) *Frequency analyses* from three consecutive days (*a*) Card ac output 3 10 l/min effect ve valve area 1 59 cm² (*b*) Card ac output 3 57 l/m n effect ve val e area 1 49 cm² (*c*) Card ac output 4 77 l/m n effective valve area 1 49 cm² Δf=frequency shift

was determined by measuring diastolic and whole beat durations on the hard copy of the frequency analys s and then performing the appropriate calculations Generally 3–4 consecutive beats from the early middle and late parts of an observation period were used for these determinations and the mean value was obtained A_e was then calculated from eq [3] us ng the mean value of the cardiac output in the observation period as Q

RESULTS

the probe was positioned so that the ultra beam traversed the region of the prosthet valve the dominant features of the audio were clicking sounds due to valve motion low frequency noise like sounds due to systol c blood velocities and a smooth whispering sound due to the diastolic blood velocities in the valve In the frequency analysis valve motion was represented as blackened vertical columns Blood velocities were represented as blackened areas situated between the representations of valve motion (Figs 1–4) The time course of the maximum diastolic frequency shift is a curve enveloping the areas representing diastolic velocities (see first diastol c period in Figs 1–4) *The disc motion in the* Bjork–Shiley and Lillehei Kaster valves was represented more distinctly than the leaflet motion in the Hancock valves Closure of the aortic valve was

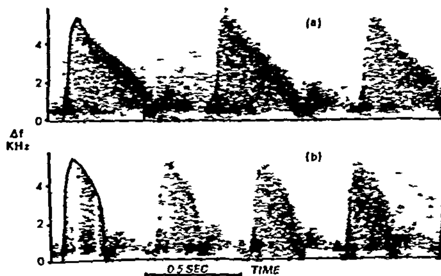


Fig 4 Patient 13 (Hancock 27) (*a*) Pulse rate 80 cardiac output 4 61 l/min effect ve valve area 1 07 cm² (*b*) Pulse rate 125 cardiac out put 4 70 l/min effective valve area 1 13 cm² Δf=frequency shift.

frequently represented as a blackened vertical column that preceded the opening of the mitral valve (Figs 1 and 3) In patients with disc valves in both mitral and aortic positions the opening and closure motions of both valves were distinctly represented (Fig 1)

The 9 patients with Bjork–Shiley valves were studied in a total of 28 observation periods (Table I) In this group A_e was 2 13±0 72 (2 S D) cm² and cardiac output 3 92±0 72 (2 S D) l/min Corresponding values for the 8 patients with Hancock valve were 1 40±0 68 (2 S D) cm² and 4 17±1 71 (2 S D) l/min (Table I) The mean A_e for each nominal valve size was 1 65 2 24 and 2 07 cm² for Bjork–Shiley 27 29 and 31 respectively and 1 24 1 39 and 1 96 cm² for Hancock 27 29 and 31 The two patients with Lillehei–Kaster valves had A_e values of 1 41 and 2 33 cm² for nominal sizes 20 and 25 respectively (Table I) The mean deviation of A_e in the multiple observation periods each patient was subjected to was ±12 9% (2 S D) and 13 3% (2 S D) for the Bjork–Shiley and Hancock valves respectively The deviation of the cardiac output in the multipe determinations performed within each observation period was ±8 6% (2 S D)

Inspection of the results disclosed no significant correlation between the value of A_e in an observation period and the values of cardiac output diastolic flow rate stroke volume or pulse rate For any given nominal valve size there was no apparent correlation between A_e and the preoperative valve lesions

The time course of the maximum systolic frequency shift was poorly defined in the frequency analyses but the peak systolic frequency shift appeared to be less than 3 KHz in all patients

DISCUSSION

Bjork et al (1) and Book (2) studied 24 patients with the Bjork–Shiley implant (18 patients with sizes 29 or 31 and 6 patients with size 27) using transseptal left atrial catheterization and retrograde left ventricular catheterization to obtain the pressure gradient The valve areas as calculated from the Gorlin formula were 2 6±0 84 cm² at rest and 3 3±0 99 cm² during exercise It is likely that an end-diastolic gradient was present during exercise and it is therefore appropriate to use a factor of 0 6 (5) to obtain the corresponding exercise value of A_e The use of this factor yields A_e=1 98±0 59 cm² during exercise The latter value agrees reasonably well with the results of the present investigation (A_e=2 13±0 72 cm²)

The validity of Torricelli s law (eq 1) is a prerequisite in the correct application of the method used in the present investigation The law is generally valid for short obstructions if the Reynolds number is sufficiently large Previous studies (5) have indicated that the law is valid for the flow in Bjork–Shiley and Lillehei–Kaster implants and it is reasonable to expect it to be valid in the Hancock implant as well Peroperative simultaneous measurements of the pressure gradient and blood velocities in a Hancock valve in the aortic position have confirmed this expectation (unpublished) It is therefore reasonable to expect the results of the present investigation to be of the same order of reliability in all three valve types studied

In a study (4) of 10 adult patients with mitral stenosis but without mitral insufficiency conducted about 18 months prior to the present investigation the values of A_e ranged from 0 59 to 2 88 cm² The hospital records of the 10 patients were reviewed in conjuction with the present investigation and it was found that 4 of the patients had undergone mitral valve replacement surgery The preoperative values of A_e in the 4 patients were 0 99 1 23 1 39 and 1 60 cm² A comparison of these preoperative values and the values of A_e determined in the present investigation demonstrates that the flow obstruction in prosthetic mitral valve implants is frequently considerable

The A_e of an implant is useful as a measure of the flow obstruction only if it is relatively independent of parameters such as cardiac output and pulse rate The present results demonstrated no significant correlation between the value of A_e and parameters such as cardiac output pulse rate etc At first sight these findings seem to be at variance with those of Bjork et al and Book who found the valve area to increase during exercise It has been demonstrated however that the Gorlin formula tends to underestimate the valve area when the gradient vanishes before the end of diastole (5) and this phenomenon may account for the findings of the above authors The variations found in the value of A_e in any given patient in the present investigation may thus be due to method error and not to changing hemodynamic conditions

Insufficiency in the implanted valve will effect underestimation of A because the actual mitral

flow rate will be higher than that predicted from the cardiac output The efficacy of the method used in the present investigation to detect and quantify implant insufficiency has not yet been established Theoretically one would expect implant insufficiency to be represented as large systolic frequency shifts in the frequency analyses In the present study the systolic frequency shifts were small (less than 3 KHz) and compatible with the frequency shifts due to blood velocities in the left ventricular outflow tract

A follow up study of the patients included in this investigation seems warranted as it would provide information about the durability of the various types of prosthetic mitral valve implants Preferably the follow up study should include collection of ultrasound manometric and angiographic data so that the ultrasound method can be evaluated more fully

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Quantitation of the Gain in Mortality and Life-Time after Pacemaker Treatment

Sven Åke Forsberg

From the Medical Department Boras Hospital Borås and the University of Goteborg Goteborg Sweden

ABSTRACT A total of 140 patients given permanent pacemaker 1963–73 were followed during three years with regard to survival Indication for pacemaker implantation was AV block in 82% and disturbed consciousness in 84% The first half of the series had a survival equal to that of the second half The survival was 86 73 and 64% and the excess mortality which is above normal was 9 16 and 20% after one two and three years respectively Concepts of fractional life time and death time were developed These express the proportion of the total observation time that is life and death respectively The fractional life time was 0 80 and the fractional death time 0 20 The excess death time was 0 12 (12%) of the total observation time Mortality, survival, life time and death time were compared in three studies, the present one and two from Denmark The great similarities were obvious An estimation of the unpaced survival during three years, based on studies of AV block before the pacemaker era, indicated the great benefit of pacemaker treatment

The purpose of the present investigation is to describe a series of patients given pacemaker and to present mortality and survival data in a way that permits comparison with other investigations before and after the era of pacemaker treatment

STUDY BASE AND METHODS

The patient series which has been collected from a central county hospital includes all the 140 patients given a permanent pacemaker from 1963 until May 31 1973 Permanent defines a pacemaker functioning until the end of the observation time which for all patients was exactly three years after implantation or until death if the patient died during the observation time

Before 1970 16% of these patients and thereafter 6% were referred to a university clinic for pacemaker implantation due to expected or actual technical difficulties or need for atrio-synchronized pacemaker With few exceptions the patients were given a pacemaker from Siemens Elema mostly ventricular triggered after the era of only fixed rate pacemaker

The reasons for pacemaker implantation were those generally recommended with one exception Acute myocardial infarction has since about 1970 constituted a relative contraindication for pacemaker treatment which means that pacemaker was not given until medical treatment had failed In some of these cases pacemaker treatment was only temporary Among the 140 patients who received permanent pacemaker 82% had AV block II or III 13% supraventricular bradyarrhythmia 1% tachyarrhythmia and 3% undefined arrhythmia with syncope Attacks of disturbed consciousness had occurred in 84% of the patients and feebleness or heart decompensation were reasons for pacemaker treatment in the majority of the remainder

The clinical diagnoses at the time of implantation are listed in Table I Criteria for coronary heart disease (CHD) were set retrospectively so that the patients at the time of implantation should show either conventional signs of a present or past myocardial infarction or have angina pectoris in the absence of other organic heart disease than CHD The diagnoses sarcoidosis systemic lupus erythematosus and muscular dystrophy are possible *but not proven causes of the heart disease* *Patients with* hypertension and absence of explicit signs of CHD were assigned to the group of undefined cardiomyopathy Under these conditions the proportion of patients with the diagnosis of undefined cardiomyopathy is as high as 64%

Among 201 patients given pacemaker between 1963–76 there were 21 who had died with the clinical diagnosis of undefined cardiomyopathy at the time of implantation The autopsy reports from these patients were examined

Further computations on the material deserve comments Fig 1 shows the survival curve for the whole series On the X axis are fractions of the observation time which is three years for every patient On the Y axis are fractions of the total number of patients observed the scale being the same on both axes The surface of the square in the figure is 1×1=1 an index of the total observation time (140×3=420 years) With such a mode of presentation the surface below the survival curve which can be calculated is expressed by a fractional number That number is here called the fractional life time or F_L which is the fraction of the total observation time that the patients were alive F_L multiplied by the total observation time gives the total life time of the subjects during three years Consequently the surface above the survival curve equals one minus F_L and that number is here called the *fractional death time or F_D. This is the fraction of the total* observation time that the patients were dead F_D multi

Table I *Clinical diagnoses in the total series of 140 patients in the 50 patients who died and in 25 patients without AV block*

	All pats		Dead pats		No of pats without AV block
	No	%	No	%	
Coronary heart disease		20		34	7
Acute myocardial infarction	11		7		
Other manifestations	17		10		
Valvular disease	17	12	10	18	4
Sarcoidosis	3		1		
Systemic lupus erythematosus	1		1		1
Muscular dystrophy	*1*				*1*
Undefined cardiomyopathy	90	64	21	42	12

plied by the total observation time gives the total death time during three years

Fig 1 also includes the expected survival curve of a hypothetic normal series matched by age and sex The figures were calculated from the official Swedish life table (9) F_L and F_D were calculated together with the survival after one two and three years The standard deviation of points on the curves in Fig 1 was calculated according to Greenwood s estimate (1) *In addition Fig 1 gives the* survival curve of 193 patients with AV block treated medically and followed during one year by Johansson (5) shortly before the era of pacemaker treatment His curve has beeen extrapolated to the three year survival figure estimated from other studies (12)

In order to study whether pacemaker treated very old patients have an excess mortality and death time deviating from the corresponding figures in younger patients the following calculations were made The series was divided into two groups of 70 patients each the young (mean age 63 maximal age 70 years) and the old (mean age 78 minimal age 71 years) The mortality and fractional life time during three years were calculated for both groups and for two hypothetic normal groups with a similar sex and age distribution from the official Swedish life table (9)

In comparisons with results of other studies data have been taken from the texts or tables or have been estimated from the figures Tests of significance were made on a level of $p<0.05$

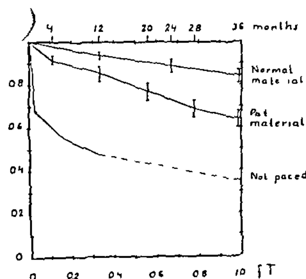


Fig 1 Survival curve of patients who received permanent pacemaker 1963–73 and the corresponding expected curve of an equal group of normal individuals and an estimated survival curve of an unpaced patient series

RESULTS

Table II shows the distribution by age in the total *series and Table III the number of patients* mean age and sex ratio in the total series as well as among those who died within three years The mean age did not differ between the total series and those who died Table I shows a higher mortality among those with a clinical diagnosis of CHD than among those with undefined cardiomyopathy The autopsy study showed that 20 of 21 patients had had AV block Fourteen patients had macroscopic changes of ischemic type in the myocardium in the presence of obstructive coronary artery disease without any *other visible organic heart disease*

The mortality in the group with arrhythmia other than AV block was similar to that in the whole series and these patients were therefore not always separated in discussions regarding AV block only

The survival curve of the first 70 patients was compared with that of the last 70 patients to get a pacemaker Tested with the χ^2 method there was no difference between the two curves at any point

In the total series the survival after one two and three years was 86 73 and 64% respectively The excess mortality *which is that above what is ex*pected in a corresponding group of normal individuals was 9 16 and 20% after one two and three

Table II *Age distribution of the total series of 140 patients*

Age (y)	Males	Females
40–44	1	0
45–49	3	1
50–54	3	1
55–59	5	5
60–64	12	1
65–69	17	15
70–74	19	9
75–79	18	3
80–84	12	4
85–89	7	3
90–94	1	0

years all figures constituting significant differences

The mortality was highest during the first four months after implantation as shown in Fig 1 and in Table IV During the first four months it was 4 6 times higher than normal but decreased thereafter and was after 28 months 1 5 times higher than in the normal group For the average paced patients the calculated probability of dying during the 29th–36th month is 0 07 compared to an expected normal value of 0 04

The fractional life time was 0 80 after three years and thus the fractional death time 0 20 implying that out of the total observation time 80% was life and 20% death In the normal group the expected fractional life time was 0 92 and fractional death time 0 08 In the patients the excess death time and consequently deficit life time was (0 92–0 80)=12% of the total observation time

The fractional life time calculated from the estimated curve in Fig 1 representing unpaced patients is 0 5 which should be compared with 0 80 in the present patients and 0 92 in the normal group The excess fractional death time in unpaced patients should be of the order of 0 9–0 5=0 4 or 40% of the total observation time

The excess death time among unpaced patients compared with the present paced patients is (0 8–0 5)×100=30% of the total observation time The gain in life time from pacing thus should be of the order of 30% of the total observation time or 0 3×420 years=126 years=1512 months This means on average 1512/140=11 months per patient during three years

In the young subgroup of patients (maximal age 70 years) the excess mortality after three years was

Table III *Patients who received permanent pacemaker 1963–73 and were observed for three years*

No of pats	140
Mean age (y)	70 5
Fraction of men	0 70
Fraction of dead pats	0 36
Mean age of dead pats (y)	69 9

23% and the excess death time 13% In the old subgroup (those above 70) the corresponding figures were 17% and 11% 17% being significantly lower than 23% in the young subgroup

DISCUSSION

In the study by Friedberg et al (4) and in four other publications quoted by them CHD was regarded as the commonest clinical diagnosis in patients with AV block With the strict criteria for CHD used in the present study that diagnosis applies to only 20% but inferring from the separate autopsy study where 2/3 of patients with the clinical diagnosis of undefined cardiomyopathy had visible CHD one can presume that a large proportion of the patients with undefined cardiomyopathy in the present study really had CHD Therefore the distribution of

Table IV *Data on mortality survival (% of the total series) fractional life time and fractional death time in paced patients from Borås Sweden and Odense Denmark (11) observed for three years compared with normal groups with similar sex and age distribution*

	Borås		Odense	
	Pats	*Normals*	*Pats*	*Normals*
Average mortality/year	11 9	5 2	10	6
Mortality during 1st 4 months	7 9	1 7	–	–
Mortality during 1st year	14 3	5 0	17	6
Survival after 1 year	86	95	83	94
Survival after 2 years	73	89	77	89
Survival after 3 years	64	84	71	83
Fractional life time	0 80	0 92	0 82	0 92
Fractional death time	0 20	0 08	0 [illegible]	[illegible]

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The Relationship between QT Interval and Ventricular Arrhythmias in Acute Myocardial Infarction

Staffan Ahnve Torbjorn Lundman and Mansour Shoaleh var

the Department of Medicine Serafimerlasarettet at Karolinska Institutet ckholm Sweden and Isfahan University Medical School Isfahan Iran

ABSTRACT Out of a total of 947 patients admitted to the CCU at Serafimerlasarettet during 2 years, all those with AMI and ventricular fibrillation (VF) or ventricular tachycardia (VT) during the CCU stay were selected The QT interval could be measured in 15 patients with VF and 12 with VT before the event The QT interval was also measured in two control groups one consisted of 27 consecutively admitted patients with AMI without ventricular arrhythmias (VA), the other of 27 non AMI patients treated in the CCU Most patients in the group with VA showed pathologically prolonged QT intervals and there were statistically significant differences between this group and the control groups regarding corrected mean QT intervals If these findings are confirmed QT measurements might be of value in the prediction of malignant VA in AMI

An ECG pattern with a long QT interval is known to be associated with ventricular arrhythmias (VA) Such ECG changes can be hereditary (2 7 11 16) or due to a variety of factors such as drugs (10) or electrolyte imbalance (15) Also increased sympathetic activity with an increase in serum catecholamines has been proposed (3 12 14) We have therefore investigated the relationship between QT interval and malignant VA in selected groups of patients admitted to a coronary care unit (CCU)

PATIENTS

Relevant clinical data on patients admitted to the CCU at Serafimerlasarettet are continuously fed into a computer and stored on tape (4 13) Out of a total of 947 patients admitted in 1975 and 1976 all those with acute myocardial infarction (AMI) and ventricular fibrillation (VF) or ventricular tachycardia (VT here defined as 10 or more consecutive ventricular ectopic beats with a rate of more than 150/min) were selected In 5 of the patients with VA no ECG tracing before the event was available and they were thus discarded Patients with left right or complete bundle branch block were excluded but patients on medication with digitalis or β receptor blocking agents were included No patient was on quinidine or procainamide treatment Thus there remained 15 patients with VF and 12 with VT to be studied

To this group of patients a control group of consecutively admitted patients with AMI but without the above mentioned VA was selected from the same data base A second control group consisted of age matched non AMI patients without a history of ischaemic heart disease admitted to the CCU for suspected AMI (1)

METHOD

From the ECG recorded on admission with a paper speed of 50 mm/sec the QT interval was measured from the onset of the QRS complex to the end of the T wave The mean of 3–6 beats in leads I–III V_2–V_4 was determined Heart rate was calculated by averaging RR intervals from the same strip Corrected QT intervals (QT) were calculated by the formula of Bazett $QT_c = \frac{QT}{\sqrt{RR}}$ (5) In a subsample two observers independently measured the QT intervals and there was no statistical difference between the two Out of 24 observations the mean QT difference between them was 0 005 sec

The significance of differences between the mean QT from each patient group was tested by Student s *t* test

RESULTS

Table I gives some descriptive data on the patients in the three groups There were no differences between the two AMI groups with and without VA regarding past history or medication except digitalis therapy which might diminish the QT interval As could be expected the control group of non AMI patients differed in many respects Serum electrolyte concentrations were analyzed on admission and no significant differences were found between

Table I *Previous diseases and treatment before admission in the three patient groups with and without ventricular arrhythmias (VA)*

	AMI without VA (n=27)	AMI with VA (n=27)	Non AMI without VA (n=27)
Males	22	21	17
Females	5	6	10
Age (y) (mean ± S D)	60±5	68±12	60±7
Previous diseases			
Hypertension	6	8	3
Previous AMI	10	17	0
Angina pectoris	14	17	0
Diabetes	5	0	1
Heart failure	6	17	0
Treatment before admission			
Digitalis	4	17	0
Diuretics	8	14	0
β blockers	0	5	0

the three groups They were all within normal limits

The mean QT_c in the three groups are shown in Table II The observed mean QT_c of 0 46 in the *AMI group with VA is pathologically prolonged and* differs significantly from both control groups

DISCUSSION

The association between prolonged QT interval and VA has been discussed in various situations which ay be grouped into two categories The first one) in the form of hereditary prolonged QT interval ncluding the Ward Romano-Barlow syndrome (2 11 16) and the Jervell Lange Nielsen syndrome (7) *The second category includes an acquired form of* prolonged QT as caused by drugs e g quinidine, ajmaline following infusion of epinephrine and due to electrolyte imbalance (12 14 15) Increased in *cidence of VA and sudden death have been ob-* served in many of these cases (10) In some of them the VF appears in the form of torsade de pointes (9) One theory is that the prolonged QT interval with delayed repolarization and development of VA sometimes may be the result of increased sym pathetic activity Sjöstrand (14) demonstrated the effects of epinephrine infusion on the ECG and he concluded that with this infusion the ST segment will shorten and because of delayed repolarization process the T wave will occupy an increasing part of the diastole *Catecholamines have been shown to* be a potent cause of VA after experimental infarc tion There is also an association between urinary catecholamine excretion and the incidence of VA after AMI (5 7)

Prolonged QT interval VA and serum catechol *amine level seem to be interrelated at least under* certain conditions The present finding of a relation ship between prolonged QT interval and VA in pa tients with AMI is consequently expected How ever it is astonishing that this relationship has not *been evaluated in previous studies on the predict* ability of primary ventricular fibrillation in AMI One reason for this neglect may be that the QT measurement is unreliable In the present study however this error was found to be of a very little importance The present study deals only with VA in the form of VF and severe VT It remains to be seen whether a relationship also exists between QT interval and other forms of ventricular tachycardias or ventricular ectopic beats Also the time relation ship between the prolongation of QT and VA should be looked for

Table II *Corrected mean QT values (QT) for the three patient groups with and without ventricular arrhythmias (VA)*

	n	QT_c (sec) (mean ± S E)	
AMI without VA	27	0 42±10	$p<0\ 005$
AMI with VA	27	0 46± 8	
Non AMI without VA	27	0 41± 4	$p<0\ 001$

CONCLUSION

A group of patients with AMI and VF or VT showed pathologically prolonged QT intervals in the ECG taken before these arrhythmias were recorded Prospective studies are needed to confirm and extend these results

ACKNOWLEDGEMENT

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Alpha-Methyldopa and Drug Fever

A Study of the Metabolism of α-Methyldopa in Patients and Normal Subjects

K Valnes [1] L Hillestad [2] T Hansen and E Arnold

From the Medical Department Sentralsykehuset i Akershus Nordbyhagen Norway and the Biological Department Dumex Laboratories Copenhagen Denmark

ABSTRACT The metabolism of α methyldopa was studied in 5 patients with febrile reactions to the drug and compared with the metabolism in 5 patients without such reactions and in 4 normal subjects A depression of the drug metabolism was found in drug fever patients, which may affect either the intestinal mucosal conjugation of the drug or the hepatic transformation The decreased metabolism is assumed to be a possible causative mechanism of the adverse drug reaction

Febrile reactions due to α methyldopa therapy have been reported by several authors They usually occur 1–3 weeks after initiation of the therapy in 1–2% of the patients (5 6 8 16) The mechanism underlying this febrile reaction is not entirely clear It has been considered to be an allergic reaction (7) but some authors have suggested the possibility of a toxic leucocytolysis as the causative mechanism (15)

The present investigation is a comparative study of the metabolism of α methyldopa in patients with and without febrile reaction to the drug

STUDY POPULATION AND METHODS

Three groups of subjects were compared (Table I)

The drug fever group (group I) consisted of five hypertensive patients who were admitted to hospital because of fever of unknown origin Their average age was 65 years Some had repeated febrile episodes followed by several diagnostic procedures before α methyldopa was thought of as a possible cause The challenge test with the drug was carried out with the patient s consent after information about the possibility of provoking complaints Two patients suffered from diabetes mellitus In connection with the febrile reaction the liver function tests were abnormal in three patients and one patient had eosinophilia These laboratory values were normalized a few days after discontinuation of the therapy

The drug fever occurred 1–3 weeks after the onset of treatment Until then all patients were given 250 mg α methyldopa three times daily

Group II consisted of five hypertensive patients treated with α methyldopa without febrile reactions Their average age was 50 years Three of the patients had used the drug for 3 weeks one for 4 months and one for 2 years In all patients the maintenance dose was 250 mg three times daily

Group III comprised four healthy volunteers Their average age was 33 years

All patients and volunteers had normal renal function and the liver function tests were normal at the time when the metabolic study was started In group I the study was performed 2–10 weeks after the last dose of α methyldopa in group II 2 weeks after discontinuation of the treatment

All subjects in the three groups were given an oral dose of 250 mg α methyldopa (Dopamet®) The renal excretion of the drug was determined in the 0–4 4–8 and 8–24-hour urines The serum concentration of α methyldopa was determined 2 4 8 and 24 hours after administration of the drug In group III only the urinary excretion was examined

Urine and serum samples were stored at −18°C until analysis The concentrations of α methyldopa in serum and urine were assayed spectrophotofluorometrically according to a modified method of Sjoerdsma et al (13) and Schlossmann et al (12)

The concentrations of total and unconjugated α methyldopa were measured and the differences between the measurements were calculated as acid labile conjugates The method described in detail by Myhre et al (9) is specific for compounds able to be transformed into fluorescent trihydroxyindole derivatives An intact catechol nucleus is required as well as a side chain with a reactive group at a position suitable for ring closure Any

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[1] *Present address and address for reprints* Medical Department IX Ullevål Sykehus Oslo Norway

[2] *Present address* Medical Department Telemark Sentralsykehus N 3900 Porsgrunn Norway

Table IV. *Urinary excretion of unconjugated (UC) and acid labile conjugated (A-LC) α-methyldopa following oral administration of 250 mg to hypertensive patients with (group I) and without (group II) drug fever caused by α-methyldopa and healthy volunteers (group III)*

	α methyldopa in urine (mg)								% of dose 0–24 hours	
	0–4 hours		4–8 hours		8–24 hours		0–24 hours			
	UC	A-LC	UC	A-LC	UC	A-LC	UC	A-LC	UC	A-LC
Group I (*n*=5)										
Mean	26.3	14.6	17.2	19.3	15.7	18.2	54.0	52.0	21.7	20.8
S.D.	6.7	13.3	4.5	8.5	5.3	5.7	8.2	22.7	3.3	9.1
Group II (*n*=5)										
Mean	10.3	12.7	9.0	18.6	1.5	11.6	20.7	42.8	8.3	17.1
S.D.	2.1	4.2	4.1	9.6	2.9	15.1	7.3	27.4	2.9	11.0
Group III (*n*=4)										
Mean	13.2	18.7	4.9	17.6	1.2	8.0	19.2	44.2	7.7	17.7
S.D.	5.2	11.4	0.8	10.1	0.9	2.5	4.8	21.9	1.9	8.8

present study puts the question whether there are differences between drug fever patients and other groups as to drug absorption, elimination or metabolism.

No significant differences were seen in total drug concentration in serum between the hypertensive groups with and without drug fever. The concentrations of unconjugated drug in serum, however, were elevated in the drug fever patients. The same result was obtained by comparing the areas under the serum level curves. Moreover, the urinary excretion of unconjugated drug was higher in the drug fever group than in both the hypertensive and the normal group. These findings indicate the existence of some difference between the groups, in either absorption, excretion or metabolism.

α-Methyldopa is excreted in the urine as both untransformed drug and its conjugates. A major urinary metabolite, detectable by the spectrophotofluorometric method, is the mono-O-sulfate accounting for about two thirds of the chemically determined urinary products. This metabolite is not formed when the drug is administered i.v., and it has been suggested that this conjugation takes place in the intestinal mucosal cells (11), i.e. before absorption of the drug as such. The 3-O-methyl-derivative, and neutral and basic fractions are found in smaller amounts in the urine (1, 2, 10). An extrarenal mechanism of drug elimination has previously been suggested (9), but recent investigations have shown that the only way in which α-methyldopa is eliminated is by urinary excretion (14).

More recent investigations (1, 14) applying radioactively labelled α-methyldopa, have shown the presence of considerable amounts of other metabolites not detectable by the usual spectrophotofluorometric method. The nature of these metabolites has yet to be cleared up, but as they occur after both oral and i.v. administration, their site of formation could be the liver.

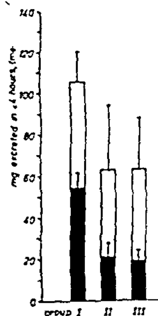


Fig. 3. Excretion of α-methyldopa in 0–24 hour urine following oral administration of 250 mg α-methyldopa to three groups of subjects. □ = Total drug, ■ = unconjugated drug. Groups I, II and III as in Fig. 2.

In the present material the rates of urinary excretion of both drug and acid labile conjugate seem to be normal or even higher in the drug fever group and failure of elimination is thus not a likely causative mechanism of the adverse drug reaction

The composition of the 0–24 hour urine shows that the relative conjugation in the drug fever group is reduced The absolute figures however show that the amount of conjugate in urine is the same as in the urines of the other groups of the administered dose 17–20% is transformed into acid labile conjugates

The absorption of an oral dose of α methyldopa in normals was found by Stenbæk et al (14) to be 43% including all metabolites The total urinary output of free and conjugated drug in group I in our study is 42% in contrast to 25% recovered in the urines of groups II and III These discrepancies can reflect two situations The absorption of drug in group I may be increased the hepatic metabolism of α methyldopa would then be normal but the relative mucosal conjugation depressed Or the absorption of drug could be normal the hepatic metabolism impaired and the mucosal conjugation normal or impaired The present study does not allow any conclusions as to which of these mechanisms are operative but both situations imply that the patient metabolizes α methyldopa to a lesser extent than the other groups This decreased metabolism can affect either the mucosal conjugation or a hepatic transformation

Whether the latter situation would be a result of an adverse drug reaction or a causative mechanism may be discussed since α methyldopa may be hepatotoxic in some patients Serum GOT was slightly elevated in three of the drug fever patients during the initial treatment with α methyldopa However at the time of the study the liver function tests were—and remained—normal indicating a primary hepatic defect

Other authors have suggested that a toxic leucocytolysis induces the pyrexic reaction in those drug fever patients (15) The present study gives no information concerning this question but a depressed drug metabolization could lead to toxic levels of α methyldopa in serum or in the cells The antihypertensive properties of the conjugates or other metabolites have not been thoroughly investigated although it has been shown that the mono-O sulfate under certain conditions has an antihypertensive effect (2)

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Massive Doses of Procainamide for Ventricular Tachyarrhythmias due to Myocardial Infarction

Ingrid Mattiasson Arne Hanson and Bengt W Johansson

From the Heart Section and Section of Toxicology Malmo General Hospital Malmo Sweden

ABSTRACT Three patients are described in whom malignant ventricular arrhythmias appeared in connection with a reinfarction some days after hospitalization for an acute myocardial infarction and in whom massive doses of procainamide up to 7 5 g/day i v were necessary to prevent these arrhythmias The serum concentration of procainamide was 2–4 times higher than the recommended upper level, but no side-effects were observed With the dose given, one would have expected still higher serum concentrations Several reasons for this finding are discussed, including the effects of renal function, intestinal leakage, storage of the drug in tissues and hitherto unknown metabolic pathways of procainamide in patients who are slow acetylators

Since its introduction in 1951 (20) procainamide has been widely used in the treatment of ventricular arrhythmias A beneficial effect of the drug has been claimed in close to 80% of the cases with ordinary dosages i e up to 1 g i v (17) It has been argued that a larger dose might result in a still higher percentage of successfully treated patients (17) A few cases have been reported in whom the usual dosage was increased 4–5 fold and this proved effective whereas the usual dose did not (3 6 8 12 21 22 23 24 27) Only in one case was the serum concentration of procainamide determined (3)

The aim of this paper is to report three patients with serious ventricular arrhythmias caused by an acute myocardial infarction who all responded well to massive doses of procainamide whereas ordinary doses were uneffective and in whom plasma concentrations were determined during treatment for their ventricular arrhythmias in the Coronary Care Unit (CCU)

CASE REPORTS

Case 1

Male born in 1917 who had been treated in 1969 with cytostatic drugs and given radiological therapy because of a malignant lymphogranulomatosis Regular check ups did not reveal any signs of Mb Hodgkin

On April 26 1975 central chest pains appeared after digging in his garden Persisting pains took him to the emergency ward where ECG showed signs of an inferior myocardial infarction S-ASAT was initially 9 27 μkat/l and rose to 17 1 μkat/l (normal value <0 67) S ALAT rose to 2 05 μkat/l (normal value <0 67) and S-LD to 58 5 μkat/l (normal value <8 0) with an increase of isozymes I and II indicating myocardial damage During his first days in the CCU some ventricular premature beats (VPB) were successfully treated with lidocaine i v and a short episode of atrial fibrillation (AF) was followed by sinus rhythm after digitalization

Mobilization of the patient started and the course was uneventful till May 7 11 days after the onset of symptoms when he suddenly became unconscious ECG showed a ventricular tachycardia (VT) passing into ventricular fibrillation (VF) After cardioversion lidocaine was given i v and later as infusion Despite lidocaine in high doses up to the limit of side-effects another episode of VF appeared as did episodes of VT The VF was successfully cardioverted while the VTs disappeared spontaneously Seven hours after onset of VF–VT multiple VPBs appeared and as they showed signs of increasing malignancy 5 mg of practolol i v was given but with no effect The lidocaine infusion continued After 100 mg of procainamide i v the VPBs disappeared immediately but returned after 20 min and again disappeared after i v procainamide During the following 60 hours increasing i v doses of procainamide were administered to cope with bouts of malignant VPBs and VTs of up to 10 min duration which disappeared immediately after i v administration of procainamide (Fig 1*a*)

In view of a high i v dose of the drug (6 5 g) on May 8 an infusion of procainamide in glucose was started on May 9 corresponding to 291 mg/hour (7 g/24 hours) No further malignant arrhythmias were observed during the infusion and after a gradual decrease of the i v infusion dosage oral administration 1 g q i d was started on May 15 The

PROCAINAMIDE
mg
600
400
200
0
— i v
ORALLY

CONC
mg/l
20
16
12
8
4
0

VT

VPB

13/2 14/2 15/2 16/2 17/2 18/2 19/2 20/2 2 /2
TIME
c

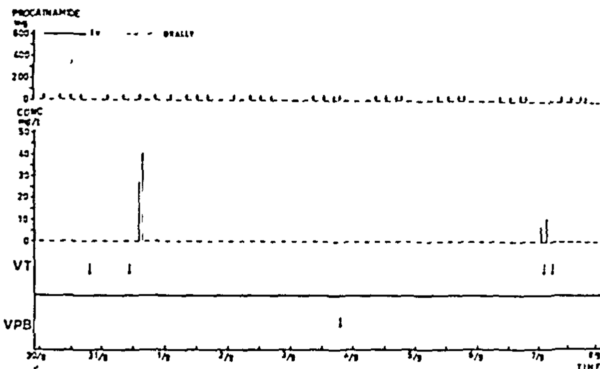
PROCAINAMIDE
mg
600
400
200
0
— v
ORALLY

CONC
mg/l
50
40
30
20
10
0

VT

VPB

22/8 23/8 24/8 25/8 26/8 27/8 28/8 29/8 30/8
TIME
d



lidocaine infusion had continued during the whole acute phase and was withdrawn on May 12. S-digoxin on May 9 was low 0.4 mg/ml (1). After May 7 S-ASAT rose from 0.67 to 0.95 μkat/l which might be due to a reinfarction.

Serum concentration values determined according to a method described earlier (13, 15) were not very high as shown in Fig. 1a and Table I. Apart from a slight degree of blurred vision no side-effects appeared. No drop in BP occurred and the Q–T duration in the ECG was not prolonged.

Acetylation test performed with isoniazid (INH) showed T½ INH = 3.4 h, which means that the patient was a slow acetylator (13). For this reason, because of a high risk for the development of systemic lupus erythematosus, procainamide was changed to mexiletine. ECG monitoring showed a stable rhythm during mexiletine administration and the patient was discharged six weeks after the initial symptoms. Cardiac decompensation appeared and he died six months later in an intractable heart failure.

Autopsy showed moderate coronary atheromatosis, partly organized thrombi in the circumflex branch of the left coronary artery and a large organized infarct in the posterior wall of the left ventricle with a papillary muscle rupture. No evidence of Hodgkin's disease. No signs of kidney disease.

Case 2

Male, born in 1908, with no previous history of heart disease who was suddenly taken ill on Feb. 5, 1975 with central chest pains radiating to his left arm. ECG showed inferior infarction. S-ASAT rose to a maximal value of 11.00, S-ALAT to 5.00 and S-LD to 30.3 μkat/l, the latter with a preponderance of isoenzymes I and II. Repeated episodes of AF during the first day in hospital required digitalization and episodes of VPBs were successfully treated with lidocaine. On Feb. 7 the lidocaine infusion was discontinued. Five hours later VT started and disappeared after a bolus dose of lidocaine. The lidocaine infusion was started again, initially at a rate of 4 mg/min, later at a lower rate because of signs of lidocaine intoxication with tremor and slurred speech. During the following day repeated attacks of VT appeared. After some of these attacks 100 mg procainamide was given i.v. with a good result. On a dosage of 200 mg five times per day i.v. only one bout of VT appeared and responded well to an additional dose of the drug. The condition seemed to stabilize and after a gradual dose reduction all antiarrhythmic therapy was discontinued on Feb. 10.

On Feb. 11 VPBs appeared suddenly and progressed to VT and VF. After four cardioversions the VF disappeared. An infusion of lidocaine was started again. During the following 48 hours three bouts of VF and repeated bouts of VT appeared, requiring five electroversions and lidocaine until toxic side-effects occurred. Procainamide i.v. stopped the VT, but it soon started again. The longest VT lasted for 80 min and was stopped by procainamide 400 mg i.v. after two electroversions had failed. During Feb. 13–16 procainamide was given in i.v. doses of 4.9, 4.8, 4.8 and 4.8 g, respectively. Serum concentration determinations showed a maximal value of 19 mg/l (Fig. 1b and c and Table I). All side-effects disappeared after the lidocaine dose had been reduced.

During this treatment the arrhythmias vanished and when the condition had stabilized, the procainamide dose

Table I *Daily i v dose and urinary excretion as well as serum concentrations of procainamide in patients 1 and 2*

Date	Procainamide		Urinary excretion/day (mg)		
	Daily i v dose (mg)	Serum concentration (mg/l)	Procainamide	Acetylated procainamide	Total
Case 1					
May 8	5 700				
May 9	7 486	15–16			
May 10	5 739	12–11	7 050	1 700	8 750
May 11	4 290	10	4 690	1 160	5 850
May 12	2 106	2– 4	2 980	820	3 800
May 13	2 522	5	1 340	330	1 670
May 14	2 200	4	1 370	330	1 700
Case 2					
Feb 11	1 600	4			
Feb 12	3 300	11– 4			
Feb 13	4 800	13–10	1 470	640	2 110
Feb 14	4 800	18–16	2 360	1 160	3 520
Feb 15	4 800	19–17	2 340	1 410	3 750
Feb 16	4 600	17			
Feb 17	1 800		1 890	940	2 830
Feb 18	1 000				
Feb 19	800				
Feb 20	2 000 p o + 200 i v		690	480	1 170
Feb 21	2 000 p o				
Feb 22	2 000 p o				
Feb 23	2 000 p o				
Feb 24	2 000 p o	8– 5			
Feb 25	2 000 p o		1 070	700	1 770

Urinary pH 5 3
p o =orally

was gradually reduced and oral treatment commenced (Fig 1*b* and *c*) A day later bouts of AF appeared with a ventricular rate of up to 160/min Digitalis was ineffective Plasma concentration of procainamide was 5–8 mg/l A right heart catheterization and flow determination revealed a small stroke volume but an essentially normal cardiac output and normal pressures in the systemic and pulmonary circulations Based on these hemodynamic findings β-blockade was started It resulted in a reduction of the ventricular rate but the AF persisted

On March 14 repeated syncopes appeared ECG monitoring with a portable ECG tape recorder (14) showed bouts of VPBs and AF After a serum digoxin concentration value (1) of 0 4 ng/ml was found the digoxin dose was increased as well as the dose of the β-blocking drug ECG tape recording five days later showed only a few VPBs and no bouts of VT In addition the syncopal attacks had disappeared The S-digoxin value was still low <0 4 ng/ml

A creatinine clearance on Feb 28 showed a value of 69 ml/min/m² BSA Acetylation test on July 30 showed the patient to be a slow acetylator with $T\frac{1}{2}$ INH=4 8 h (13) Despite this he was given a maintenance dose of procainamide 0 5 g q i d The patient was discharged in good condition and remains well A slight increase in ANF titre to 1/128 was observed in July 1975 and procainamide was therefore changed to mexiletine which he is still taking The ANF titre had normalized a few months after this change

Case 3

Male born in 1910 with angina on effort since 1950 responding well to nitroglycerine and since 1969 signs of slight cardiac decompensation treated with digitalis and diuretics On Aug 10 1970 when mowing the lawn he had a short fainting spell in connection with left sided chest pains After another two episodes of chest pain he stopped working and bicycled to the emergency ward where ECG showed an anterior infarction S-ASAT rose to a maximal value of 6 76 and S-LD to 47 7 µkat/l with a predominance of isoenzymes I and II The maximal S ALAT value was 1 44 µkat/l

VPBs appeared immediately after admission They responded well to lidocaine and the lidocaine infusion was discontinued after two days On Aug 13 chest pain and VPBs reappeared but again there was a good response to lidocaine Mobilization was started and on Aug 18 an X ray of the heart showed a finding similar to one year earlier with a relative volume of 740 ml/m² BSA and a slight congestion of the lungs

On Aug 22 a VT appeared which did not respond to lidocaine. Procainamide 100 mg i.v. stopped the attack but there were frequent recurrences. During the following 30 hours repeated cardioversions were necessary to stop VTs and VFs. The amounts of procainamide given as well as the serum concentrations are shown in Fig. 1d and e. The usual moderate doses of procainamide were increased, the total amount administered i.v. during Aug 23–24 being 2800 mg. As shown in Fig. 1d and e, it was not until very high serum concentrations were obtained that the VT attacks could be stopped. The maximal serum concentration was 46 mg/l. The patient showed no side-effects and no drop in BP. This second episode of serious arrhythmias was ascribed to a reinfarction because S-ASAT rose to 1.54 and S-LD to 17.4 µkat/l.

The patient was discharged in good condition on Sept 19. Signs of cardiac decompensation appeared and culminated in a pulmonary edema which was treated successfully. His condition improved gradually and a year after the infarction he could regularly take long bicycle trips and work in his garden. In late 1972 he had a new infarction and died from an intractable VF. Autopsy showed a pronounced coronary atheromatosis, a diffuse and patchy interstitial myocardial fibrosis and multiple infarctions in the left ventricle.

DISCUSSION

It is well known that a given dose of procainamide administered orally or i.v. can result in varying serum concentrations (3, 11, 15, 18, 19). These pronounced individual variations have been ascribed to differences in degree of absorption, volume of distribution and elimination rate (18). This is well illustrated by our three cases, among whom the patient with the lowest daily dose reached the highest serum concentration.

According to previous studies there is a good correlation between serum concentration and clinical effect, and the so-called therapeutic range has been said to be 4–8 mg/l (8, 12). At serum concentrations above 8 mg/ml an increasing number of side-effects have been reported and at values above 16 mg/ml serious side-effects with conduction disturbances and deleterious hemodynamic influences have been reported (19). All of our three patients have exceeded these serum concentrations without any side-effects—in case 3 the serum level was five-fold above the recommended value.

It was apparent that these high serum levels were necessary in the acute phase to prevent a deleterious ventricular tachyarrhythmia. Our conclusion is that in the acute phase of a myocardial infarction the therapeutic range may be very broad. It is of the utmost importance to follow the effects of therapeutic interventions not only from single or repeated serum concentration values but to take the whole clinical picture into consideration, including the effect of antiarrhythmic drugs on the monitoring oscilloscope. If a drug effect judged from whether the serum concentration value is within the "therapeutic range" had been law, our three patients would no doubt have died "lege artis".

This does not mean that serum concentration values of antiarrhythmic agents are irrelevant. They certainly are of great help, especially in patients with a discrepancy between the expected and observed effect of a drug, in patients with renal or hepatic insufficiency, which affects elimination and biotransformation of the drug, and in patients with endocrine disorders, especially affecting thyroid function. In addition, determinations of serum concentration are necessary for analysis of the pharmacokinetic behaviour of a drug.

Our experience is that the recommended upper therapeutic level of 8 mg/l for procainamide is too low and we recommend a therapeutic range of 4–[illegible] mg/l. It should be remembered, however, that some patients respond to a serum concentration level below the lowest recommended value; furthermore some patients do not respond until a level much higher than the recommended value is reached, as illustrated by our three patients. It is also apparent from the case histories that the effective serum concentration varies with the phase of the disease—the highest values necessary were seen just after the onset of the myocardial infarction; later in the course of the disease lower concentrations were effective in preventing tachyarrhythmias.

The reason for this changing sensitivity in our three patients is not known. It may be due to varying sensitivity of the "procainamide receptors". It is also possible that the blood flow in the tissue around the infarcted area was so diminished that a very high serum concentration was necessary to reach a satisfactory concentration at the effector organ.

The procainamide doses given to our three patients were excessively high. What is the reason for the comparatively moderately high serum concentrations, especially in cases 1 and 2, despite long-term administration of heavy doses of procainamide? Several explanations may be discussed. Unsatisfactory absorption can be ruled out since the drug was given intravenously.

Renal function. Approximately 60% of the pro-

cainamide is excreted in unchanged form through the kidneys (19) Weily and Genton (26) observed a direct correlation between renal elimination and creatinine clearance A low clearance of 69 ml/min/1 73 m BSA was found in our case 2 Despite this large amounts of procainamide were excreted in the urine (Table I)

Changes in urine flow due to water deprivation and water excess do not influence procainamide clearance (9) A 3–6-fold increase in urine volume as a result of administration of furosemide did not change procainamide clearance (24)

Tubular reabsorption of drugs depends largely on the lipid/water partition coefficient and for ionizing drugs this depends on the pH of the urine When the urine is acid basic drugs are more highly ionized and are excreted to a greater extent than in alkaline urine The converse holds for acid drugs and the amount of drug reabsorbed is thus dependent upon the concentration gradient of non ionized drug between luminal and peritubular fluid

In the case of procainamide with pKa of 9 4 a low urine pH should decrease reabsorption and increase the amount of procainamide in urine In our case 1 urine pH was determined and found to be low 5 3 Although the importance of urinary pH has been stressed (24) others conclude that if significant passive diffusion of procainamide does take place in the kidney it is limited to the proximal portion of the nephron is not pH-dependent and is more than compensated for by active tubular secretion (9) In a previous study we could show that 8 g sodium bicarbonate orally did not influence the serum concentration values of procainamide (15) What is important is not the pH of the voided urine ut of the urine in the part of the tubules where the on ionic diffusion takes place

Intestinal leakage might account for a certain percentage of the drug disappearing from the circulation However no other signs of gastrointestinal dysfunction were observed In case 1 the amount of procainamide and its metabolite N acetylprocainamide (2 10) in the stool collected during a 24-hour period was 3 1 and 47 5 mg/100 ml respectively on May 12 Corresponding values on May 13 1975 were 3 2 and 39 5 mg/100 ml

The presence of both procainamide and N acetylprocainamide in the stool may be due to excretion through the bile or directly into the gastric juice (25) the concentration ratio juice plasma increasing with the basicity of the drug Both procainamide and N acetylprocainamide have pK_a values which would make such a transfer probable The high concentration of N acetylprocainamide is difficult to explain at present It is possible that some of the N acetylprocainamide is formed by gastrointestinal microorganisms from the procainamide secreted from plasma

Abnormal biotransformation of procainamide is a further possibility Some metabolites are known e g para amino benzoic acid and 2–10% of the procainamide is excreted through the kidneys in this form while N acetylprocainamide is responsible for about 23% (16) In our study N acetylprocainamide was determined according to the method of Elson et al (7) and constituted 20–35% of the total amount of procainamide and N acetylprocainamide found in the urine The lowest figure refers to case 1 who was a slow acetylator

Storage in the tissues Procainamide is known to be stored in some tissues e g kidneys liver lung and heart The concentration quotient between heart muscle and plasma in the dog is 3 1 (20) Storage of the drug in the tissues is not probable since large amounts of the drug were administered over a very long time (Fig 1*a b* and *c*) and in case 1 a sample of the subcutaneous adipose tissue contained no procainamide

It seems from Table I as if in case 1 the amount of procainamide administered was excreted in the urine either as unchanged procainamide or as its acetylated metabolite In case 2 however it seems as if more was administered than was found in the urine

No gastrointestinal disturbances were observed in case 2 and there is thus no reason why this patient would leak drug through the intestinal mucosa He is however a slow acetylator and it may be speculated that he may use other metabolic pathways for the biotransformation of procainamide than those already known It may be worthwhile to search for other metabolites of procainamide than the acetylated form of the drug in patients who are slow acetylators

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The Effect of Propranolol on Serum Levels of T_4, T_3 and Reverse-T_3 in Hyperthyroidism

Gunnar Kallner Jan Gustaf Ljunggren and Monica Tryselius

From the Department of Medicine St Goran s Hospital and Department of Medicine II Sodersjukhuset Stockholm Sweden

ABSTRACT The effect of propranolol (40 mg t d s) on the peripheral levels of T_4 T_3 and reverse T_3 was studied in 26 patients with hyperthyroidism The compounds were measured by specific radioimmunoassay techniques The levels were followed for five weeks and compared with the levels before treatment The results show that propranolol had no significant effect on the peripheral levels of T_4 despite a rapid amelioration of clinical symptoms A significant reduction of T_3 levels was obtained during the first to fourth week of treatment No significant decrease was obtained after the fourth week A significant elevation in reverse T_3 levels was obtained during the second to fourth week of treatment No significant change was obtained either during the first week or after the fourth week Inconsistent fluctuations in hormone levels both before and during treatment, could be seen in individual cases The results suggest that the reduction of clinical symptoms must be caused by an extrathyroidal action of propranolol which does not seem to involve the pituitary gland

The symptoms and signs of hyperthyroidism have long suggested that an involvement of the sympathetic nervous system is highly probable in their production The possibility of such an involvement is strengthened by the observations that β adrenergic blocking agents such as propranolol reduce many of the peripheral manifestations and symptoms of hyperthyroidism after a few days of treatment (1 5 9) It has also been demonstrated that both α and β adrenergic agonists have thyroid stimulating properties (7) Furthermore the demonstration of interfollicular sympathetic nerve terminals in the human thyroid adds a morphological basis suggesting a direct influence of the sympathetic nervous system on the human thyroid follicle cells (6)

The mechanism behind the clinical effects of the β adrenergic blocking agents in hyperthyroidism is unknown One of the difficulties in the elucidation of this mechanism is posed by controversial reports regarding the effect of the drugs on the peripheral hormone levels in hyperthyroid patients Decreased (2 8) increased (11) or both increased and decreased thyroid hormone levels (4) have been reported

The aim of the present investigation was to study the levels of thyroxine (T_4) 3 5 3 triiodothyronine (T_3) and 3 3 ,5 triiodothyronine (reverse T_3) by radioimmunoassay techniques during treatment of hyperthyroidism with propranolol over a period of several weeks

PATIENTS AND METHODS

Twenty six patients with hyperthyroidism were studied All had clinical and laboratory signs of the disease The age range was 25–81 years (mean 52) Twenty-one were females Nineteen of the patients had diffuse toxic goiter three multinodular toxic goiter and four solitary toxic adenoma No patient with chronic obstructive lung disease heart failure progressive ophthalmopathy or other severe disease where propranolol administration is contraindicated was included in the study

Propranolol 40 mg t d s (Inderal® Imperial Chemical Industries Ltd England) was given orally Each patient was evaluated regularly on an out patient basis with regard to the clinical features of the disease and to laboratory tests

The laboratory investigation included the determination of T_4 and T_3 by a radioimmunoassay technique recently described (3) The interassay variability for T_4 has a coefficient of variation of 7 5% and for T_3 of 11 1% Reverse T_3 was determined by a commercial kit (Hypolab S D Coinsins Switzerland) The normal levels (mean ± S D) in peripheral venous serum are 89±17 nmol/l for T_4 1 77+0 34 nmol/l for T_3 and 0 34±0 20 nmol

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in hyperthyroidism also indicates that the recently demonstrated interfollicular sympathetic nerve terminals in the human thyroid do not seem to be involved in the reduction of symptoms obtained with the drug

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Non-Invasive Assessment of Cardiac Function in Poisoning with Drugs

Oddbjorn Brubakk and Tore Kalager

From Medical Department B University Hospital Haukeland Sykehus Bergen Norway

ABSTRACT Systolic time intervals (STI) have been used in evaluating cardiac function in 21 patients who had taken an overdose of drugs Registrations were made in all patients on arrival at the hospital and on the third day On arrival STI was abnormal in 16 patients (76%) At the second registration it was still abnormal in ten (48%) Prolongations of total electromechanical systole (QS2) and preejection period (PEP) were the most frequent findings The patients with abnormal STI at the first registration showed a significant improvement in QS2 ($p<0.01$) and PEP ($p<0.05$) from the first to the third day Two of the four patients who had taken amitriptyline in a slow release form showed a prolongation of PEP and an increase in PEP/LVET from the first to the third day, and one patient had a further prolongation on the fifth day The findings may be explained by a reduction of cardiac contractility caused by the membrane-stabilizing effect of the drugs

The cardiotoxic effects produced by an overdose of tricyclic antidepressants (TCA) are well known Most often these effects have been studied by ECG (2 5 9 10 11) The main changes found in ECG are prolongation of the PR intervals widening of QRS complexes and ST T changes Cardiovascular complications have also been reported in patients taking phenothiazides or related compounds (1) Central hemodynamics have been studied in patients who had taken an overdose of TCA and hypnotics (12)

Measurement of systolic time intervals (STI) is a simple non invasive method for evaluating cardiac function in various groups of patients (4 13) This study was performed in order to follow cardiac function in patients admitted to the hospital for drug poisoning—and to compare STI and ECG changes

PATIENTS

The patients consisted of 8 women and 13 men Individual data are given in Table I

Knowledge of the drugs ingested was based on information given by the patients when they recovered consciousness It was not possible to determine the amount of drugs ingested The patients information on this point was not always reliable and in addition varying amounts of drugs were removed by gastric lavage Twelve patients were in coma and 9 were lethargic on arrival at the hospital

Initially all the patients were treated in a medical intensive care unit Electrolyte and fluid imbalances were corrected as necessary Dialytic therapy forced diuresis or respirator treatment were not used Cardiovascular drugs were not given

METHODS

ECG and BP were registered in all the patients on admission and later at set intervals

STI was measured as described by Weissler et al (13) from synchronous registrations of ECG carotid pulse wave and phonocardiogram STI was recorded on arrival at the hospital and on the third day in two patients also on the fifth and eighth days The intervals determined were total electromechanical systole (QS2) left ventricular ejection time (LVET) and preejection period (PEP) The index PEP/LVET was calculated QS2 LVET and PEP were corrected for heart rate by calculating percentages of the normal values at different heart rates The normal values were based on registrations from 50 individuals without heart disease described earlier (3)

Statistical analysis was performed with Wilcoxon s test for two samples and Wilcoxon s test for paired differences

RESULTS

None of the patients had arrhythmias that required therapy Three patients had widening of the QRS complexes (>0 10 sec) at the first registration

Table I *Individual data and systolic time intervals*

QRS=QRS complexes QS2=total electromechanical systole LVET=left ventricular ejection time PEP=preejection period

Pat no	Sex	Age (y)	Drugs	Registration[a]	BP (mmHg)	Consciousness	QRS (sec)	HR (beats/min)	QS2 (%)	LVET (%)	PEP (%)	PEP/LVET
1	♀	16	Amitriptyline (slow release) thioridazine	a	120/80	Coma	0.09	79	131	132	128	0.335
				b		Lethargic	0.09	95	115	107	138	0.440
				c		Awake	0.09	93	112	99	150	0.520
				d		Awake	0.08	69	103	102	105	0.350
2	♂	18	Flupentixol	a	150/70	Lethargic	0.08	112	96	94	103	0.380
				b		Awake	0.09	90	98	95	110	0.400
3	♂	36	Diazepam nitrazepam	a	100/70	Coma	0.08	90	99	96	109	0.385
				b		Awake	0.08	83	104	101	114	0.385
4	♂	25	Chlorzoxazone dextropropoxiphene	a	80/55	Coma	0.12	76	105	100	118	0.405
				b		Awake	0.10	55	97	93	109	0.400
5	♂	25	Promethazine levopromazine	a	95/60	Coma	0.10	130	109	101	115	0.450
				b		Awake	0.08	92	98	91	118	0.450
6	♂	43	Promethazine chlorprotixene	a	110/75	Coma	0.06	115	92	93	90	0.335
				b		Awake	0.07	99	105	104	109	0.360
7	♂	26	Amitriptyline (slow release) promethazine	a	110/80	Coma	0.12	102	117	108	143	0.465
				b		Awake	0.09	99	112	108	129	0.415
8	♂	43	Chlorpromazine	a	150/80	Lethargic	0.09	86	114	112	121	0.370
				b		Awake	0.08	83	113	113	121	0.370
9	♀	19	Chlorpromazine thiphenazine	a	130/80	Lethargic	0.09	77	104	97	127	0.450
				b		Awake	0.07	80	100	99	105	0.365
10	♂	48	Secobarbital	a	170/100	Lethargic	0.08	80	103	94	132	0.485
				b		Awake	0.07	68	98	98	119	0.470
11	♀	39	Amitriptyline (slow release)	a	140/85	Coma	0.10	119	115	106	143	0.465
				b		Awake	0.10	92	111	95	157	0.570
				c		Awake	0.08	85	105	100	122	0.470
				d		Awake	0.07	74	104	109	91	0.290
	♂	72	Meprobamate	a	210/90	Coma	0.08	110	104	92	137	0.510
				b		Lethargic	0.09	69	92	95	84	0.305
13	♂	34	Phenobarbital phenytoin	a	130/80	Coma	0.11	88	109	100	134	0.455
				b		Awake	0.09	85	108	109	104	0.355
14	♀	30	Chlorimipramine flupentixol	a	110/65	Coma	0.06	96	111	110	113	0.355
				b		Awake	0.07	63	103	105	99	0.320
15	♀	14	Dexchlorpheniramine	a	140/90	Lethargic	0.06	85	109	110	106	0.330
				b		Awake	0.06	81	101	101	104	0.355
16	♀	19	Phenobarbital	a	100/75	Lethargic	0.08	81	113	108	132	0.420
				b		Awake	0.08	88	109	104	124	0.405
17	♂	51	Trimipramine	a	130/80	Coma	0.09	86	107	95	144	0.525
				b		Lethargic	0.08	87	103	97	120	0.420
18	♀	38	Doxepin	a	135/80	Lethargic	0.07	61	106	98	131	0.455
				b		Awake	0.07	77	102	97	116	0.410
19	♂	26	Phenobarbital	a	120/80	Lethargic	0.09	75	101	102	105	0.355
				b		Awake	0.08	74	99	102	93	0.310
20	♂	26	Phenobarbital	a	140/90	Coma	0.08	108	115	116	110	0.325
				b		Awake	0.08	78	96	92	111	0.415
21	♀	23	Amitriptyline (slow release)	a	110/70	Lethargic	0.08	70	115	106	144	0.465
				b		Lethargic	0.08	72	114	105	143	0.470

[a] a=first b=third c=fifth d=eighth day

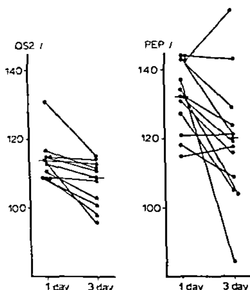


Fig 1 Total electromechanical systole (*QS2*) and preejection period (*PEP*) in patients with abnormal lengthening of *QS2* (n 11) and *PEP* (n=13) on arrival at the hospital The fall from the first to the third day was significant both in *QS2* ($p<0.01$) and in *PEP* ($p<0.05$) (Wilcoxon s test for pair differences) —=Median values

(Table I) One patient had a systolic BP below 85 mmHg none had a diastolic BP above 100 mmHg

On admission 16 (76%) of the patients had abnormal STI QS2 was prolonged (>108%) in 11 patients LVET (>110%) in three PEP (>115%) in 13 and PEP/LVET (>0 45) in eight patients None of the intervals were shortened in any of the patients at the first registration

On the second registration STI was still abnormal in ten patients (48%) QS2 was prolonged in six and LVET in one while PEP was prolonged in ten and shortened in one PEP/LVET was increased in two patients

In the patients with prolonged QS2 and PEP on admission there was a significant fall ($p<0.01$ and $p<0.05$ respectively) from the first to the second registration (Fig 1) PEP was further prolonged and PEP/LVET increased on the third day in two patients and in one patient on the fifth day (Fig 2) STI became normal in both on the eighth day These two patients had taken amitriptyline in a slow release form

In QS2 there was a significant difference ($p<0.02$) between the eight patients who had taken TCA and the 13 who had taken other drugs STI was pathological in seven of the eight patients with TCA intoxication

When STI was normal on admission it did not become abnormal at a later stage There was no significant difference in STI between the patients who were in coma and those who were lethargic at the first registration

DISCUSSION

Miscellaneous factors such as change in heart rate venous return afterload and contractility will affect STI (6) Heart rate was corrected for in all the patients Both an acute rise in arterial BP and negative inotropic agents will tend to prolong QS2 LVET and PEP and increase PEP/LVET (8)

In this study prolongation of QS2 and PEP and increased PEP/LVET were the most common findings As BP was normal in the majority of the patients these findings may be explained by the negative inotropic effect of the drugs ingested Negative inotropy is caused by the membrane stabilizing mechanism which has been shown with various groups of drugs These groups include barbiturates phenotiazine derivates TCA antiepileptic drugs and antihistamines (7) These drugs are all represented in this study

LVET was less influenced than QS2 and PEP Lengthening of LVET caused by reduced contractility may be opposed when reduction in stroke vol

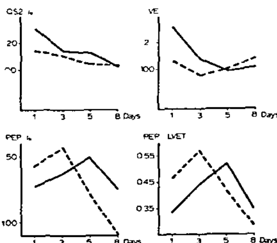


Fig 2 Systolic time intervals in two patients who had taken an overdose of amitriptyline in a slow release form *QS2*=total electromechanical systole *LVET*=left ventricular ejection time *PEP* preejection period

ume occurs at the same time The fall in QS2 and PEP from the first to the third day in the patients with abnormal lengthening of QS2 and PEP on arrival indicates improvement in cardiac function This improvement seems to occur parallel to the excretion of the drugs

Two patients who had taken amitriptyline in a slow release form showed a further reduction of cardiac performance from the first to the third and one patient even to the fifth day Cardiac function in these patients thus had to be monitored for several days

A hyperkinetic circulatory state has been shown in patients with TCA poisoning (12) However while cardiac output was normal stroke volume was in general somewhat small peripheral resistance was unchanged while $(dP/dt)_{max}$ was reduced during the comatous stage Pathologically broad QRS complexes and reduced $(dP/dt)_{max}$ might be due to electromechanical dysfunction

Widening of QRS complexes was present in only one of the patients with TCA poisoning in this study and thus cannot explain the prolongation of PEP

The patients included are a heterogeneous group with respect to type and amount of drugs ingested This may explain the varying results Heart function was affected in 76% of the patients Although one of these developed clinical signs of heart failure cardiac performance has to be closely monitored when therapy such as forced diuresis and fluid infusion is planned None of the patients had arrhythmias that required therapy and only three had widening of the QRS complexes STI was thus a far more sensitive indicator of cardiac affection than ECG in these patients The recordings can be repeated easily in order to study improvement or deterioration in cardiac function

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Effect of Alcohol on Chemotaxis, Adherence and Phagocytosis of Human Polymorphonuclear Leucocytes

Bengt Hallengren and Arne Forsgren

From the Departments of Medicine Endocrinology and Clinical Bacteriology Malmo General Hospital University of Lund Malmo Sweden

ABSTRACT In view of the presumed increased susceptibility of chronic alcoholics to infectious diseases, the influence of alcohol in vitro on leucocyte adherence, neutrophil chemotaxis phagocytosis and intracellular killing was investigated with highly standardized methods A significant inhibitory influence on these leucocyte functions was found only at alcohol concentrations in excess of those obtained clinically An interesting observation not reported before was increased adherence, phagocytosis and chemotaxis at low to moderate concentrations of alcohol

It is generally presumed that chronic alcoholics have an increased susceptibility to infectious diseases especially pneumonia (2 7 11 14 25) This increased susceptibility could be due to alcohol per se or to various associated problems in chronic alcoholism like malnutrition Chemotaxis adherence phagocytosis and intracellular killing of microbes are fundamental functions of polymorphonuclear neutrophil leucocytes Thus inhibition of leucocyte function by alcohol may have important implications for host defence against infections The possibility that the function of peripheral blood leucocytes may be affected by alcohol has been investigated earlier with conflicting results (1 3 9 10 19 20 23 24)

The purpose of the present study was to investigate with highly standardized methods whether alcohol in vitro has an influence on leucocyte adherence neutrophil chemotaxis or intracellular killing

MATERIAL AND METHODS

Leucocytes and sera

Human peripheral leucocytes from healthy laboratory personnel were used in all experiments

For chemotaxis experiments the leucocyte rich plasma was removed after the erythrocytes had been allowed to sediment by gravity at room temperature for a period of about 1 hour The leucocyte rich plasma was then adjusted to a concentration of 10 polymorphonuclear leucocytes/ml

For adherence tests heparinized venous blood was used

For phagocytosis and bactericidal tests leucocytes were prepared by dextran sedimentation of heparinized venous blood Erythrocyte contamination was removed by osmotic shock as described by Davies et al (4) The leucocytes were then washed three times in phosphate buffered saline pH 7 2 and finally suspended in buffer used for phagocytosis test

Pooled normal human serum was collected from clotted humal blood by standard laboratory techniques The sera were stored for not more than 2–3 hours at 4 C or frozen immediately at 70°C

Chemotaxis

Leucocyte chemotaxis was studied by the method of Nelson et al (16) as modified by Forsgren & Schmeling (5) Briefly agarose plates were prepared containing 0 5 ml of 10×tissue culture medium 199 (Flow Laboratories) 0 15 mmol of Hepes (N 2 hydroxyethyl piperazine N2-ethanesulfonic acid buffer) (Schwarz/Mann Div of Becton Dickinson & Co Orangeburg NY) 25 mg albumin the concentration of alcohol to be tested and distilled water to a total volume of 4 ml Finally 1 0 ml of 5% agarose (Litex Glostrup Denmark) was added The pH of the medium was adjusted to 7 4 before addition of the agarose Five ml of the agarose solution were then transferred to a tissue culture dish (60 by 15 mm 3002 Falcon Oxhard Calif) and allowed to harden Wells with a diameter of 3 mm and spaced 3 mm apart were cut (Fig 1) Each plate contained five series of three wells

Half a ml of a plasma suspension containing 10^7 polymorphonuclear leucocytes/ml was preincubated during shaking for 30 min at 37°C with 0 5 ml alcohol diluted in saline in different concentrations After centrifugation for 10 min at 200×*g* the cell pellet was resuspended in a small portion of the supernatant to give a final concentration of 10^8 polymorphonuclear leucocytes/ml Cell suspensions preincubated with alcohol were added to the middle wells in three of the five well sets in the agarose plates with the corresponding concentration of alcohol The remaining two middle wells were filled with 10 μl of

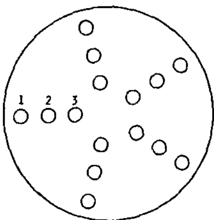


Fig 1 Tissue culture dish with agarose gel used for chemotaxis Pattern of application in wells *1*=1× tissue culture medium *2*=cell suspension with alcohol *3*=E *coli culture filtrate*

plasma containing 10^8 polymorphonuclear leucocytes/ml which had not been preincubated with alcohol The five inner wells were all filled with 10 μl of a chemotactically active E coli culture filtrate and the outer wells were filled with 10 μl of 1× tissue culture medium (Fig 1) Incubation was carried out for a period of 3 min at 37 C in a humidified atmosphere containing 5% CO_2 in air The plates were then fixed and stained and the chemotaxis was quantitated by measurement of a greatly enlarged projection of the migration patterns i e the number of mm from the wells was measured

Results were calculated as percentage of control values t were obtained using cells preincubated with tissue ture medium without alcohol and tested for chemotac tapacity in plates lacking alcohol content but otherwise ntical to the test plates

Adherence

The leucocyte adherence to nylon fibers was analyzed according to MacGregor et al (12) essentially as modified by Palmblad et al (18)

To heparinized venous blood ethyl alcohol diluted in saline was added and preincubated for 30 min One ml aliquots of the blood specimen were applied to the top of three adherence columns These consisted of Pasteur glass pipettes with a tip inner diameter of about 0 8 mm (Labora Stockholm Sweden) in which 70 mg of scrubbed nylon fibers (Fenval Laboratories Illinois USA) had been packed into a 15 mm long column Having passed the blood through the three pipettes which were incubated at 37°C it was collected in plastic tubes containing 10 μl EDTA (disodium-ethylenediaminetetraacetic acid—150 mg/ml) The number of leucocytes was counted in a Linson counter 431 The medium value for three pipettes was used and results are given as percentages of control adherence with saline instead of alcohol

Phagocytosis procedure

A method for quantitative measurement of phagocytosis by neutrophils essentially as described by Forsgren et al (6) was used Five μl of a leucocyte suspension containing 1×10^8 polymorphonuclear leucocytes/ml in Krebs Ringer phosphate medium pH 7 4 with the addition of 6% bovine serum albumin (Poviet Producten N V Amsterdam) were added to round bottomed glass tubes Five μl of ethyl alcohol diluted in saline in different concentrations were then added

The tubes were shaken in a water bath at 37 C for 30 min Five μl of opsonized lipopolysaccharide-covered radioactive oil emulsion were added to each tube The lipopolysaccharide used was extracted from E coli 0 118 by a modification of the phenol in water method of Westphal et al (26) The radioactive oil emulsion consisted of nonradioactive diisodecylphthalate (ICN Pharmaceuticals Inc Life Science Group Plainview NY) to which approximately 2% ^{3}H labelled triolein (pro-

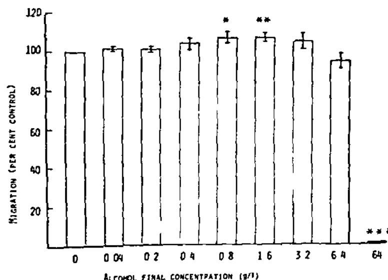


Fig 2 Chemotaxis of normal human polymorphonuclear leucocytes preincubated with alcohol for 30 min then concentrated and tested in gels containing the same concentration of alcohol (mean ± S E M) *0 01<*p*<0 05 ** 0 001<*p*<0 01 ****p*<0 001

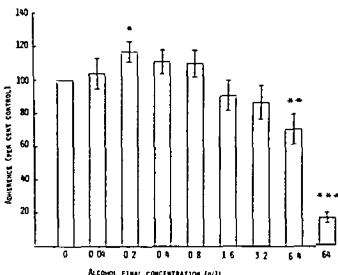


Fig 3 Influence of alcohol on human leucocyte adherence Blood and alcohol in various concentrations were preincubated for 30 min at 37°C (mean ±S E M) 0 01 $<p<0\ 05$ **0 001$<p<$0 01 * $p<$0 001

vided by Dr P Belfrage Lund Sweden) had been added After 6 additional min of shaking at 37°C the phagocytosis process was stopped by adding 3 ml of ice-cold 1 mM N-ethylmaleimide in saline The leucocytes were obtained as a pellet by centrifugation at 500 *g* for 10 min The supernatant was poured out and the inside of the tube was carefully wiped with a cotton swab The cell pellet was resuspended in 3 ml of fresh saline and the procedure was repeated The cell pellet was finally solubilized by adding 0 5 ml of Soluene (Packard Instrument Co Inc) transferred to a plastic vial to which scintillation fluid Instafluor (Packard) was added The radioactivity was measured in a liquid scintillation counter (Packard Model B 2450) Results were calculated as percentages of control values obtained for phagocytosis in the absence of alcohol All tests were carried out in triplicate

Bactericidal tests

Phagocytosis and bactericidal capacity of polymorphonuclear leucocytes were tested in the phagocytosis system of Maaløe as modified by Quie et al (21)

The leucocytes were suspended in Hank s balanced salt solution to a concentration of 10^7 polymorphonuclear per ml Ethyl alcohol diluted in Hank s solution was present during the test in different concentrations Staphylococcus aureus 502 A was used as test organism and was opsonized with pooled human serum

Statistics

Statistical analyses were made with Student s *t* test

RESULTS

Fig 2 shows the influence of alcohol on polymorphonuclear leucocyte chemotaxis in 5 experiments Ethyl alcohol in a final concentration of 64 6 4 3 2 1 6 0 8 0 4 0 2 0 04 and 0 g/l was tested There is a tendency with low or relatively high significance to increased chemotaxis at alcohol 0 8 and 1 6 g/l a decrease of high significance at 64 g/l and a tendency not statistically significant to decreased chemotaxis at alcohol 6 4 g/l

Adherence of leucocytes in the presence of alcohol in 9 experiments is demonstrated in Fig 3 Alcohol in a concentration of 0 2 g/l gives a low graded significant increase in adherence of leucocytes to nylon fibers and at alcohol 6 4 g/l or higher concentrations a significant degree of inhibited adherence was detected

Fig 4 shows a summary of 6 phagocytosis experiments with opsonized lipopolysaccharide oil emulsion and human polymorphonuclear leucocytes The influence of alcohol on phagocytosis was measured by uptake of radio-labelled emulsion There is a tendency not statistically significant to increased phagocytosis in the presence of alcohol 0 04 g/l At 6 4 g/l and higher concentrations of alcohol there is a significantly decreased phagocytosis For all other concentrations of alcohol there is no effect on phagocytosis capacity of the polymorphonuclear leucocytes neither positive nor negative

Fig 5 shows a representative experiment on phagocytosis and bactericidal capacity of polymorphonuclear leucocytes in the presence of alcohol when tested in the phagocytosis system of Maaløe With this method there is a decreased phagocytosis at alcohol 64 g/l a tendency to de

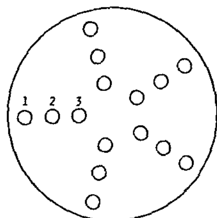


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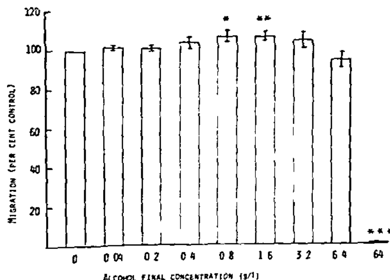


Fig 2 Chemotaxis of normal human polymorphonuclear leucocytes preincubated with alcohol for 30 min then concentrated and tested in gels containing the same concentration of alcohol (mean ± S E M) *0 01<p<0 05 *0 001<p<0 01 ** p<0 001

Our chemotaxis study is in good accordance with that of Spagnuolo and MacGregor giving no inhibition of chemotaxis below 6 4 g/l of alcohol At 6 4 g/l there is a tendency although not statistically significant to decreased chemotaxis and at 64 g/l chemotaxis is completely inhibited In addition our study indicates a statistically significant increase of chemotaxis at alcohol 0 8 and 1 6 g/l However the increase was low and probably not of clinical significance

MacGregor et al (12) have shown a decreasing granulocyte adherence to nylon fibers with successively increasing concentrations of alcohol testing 1 g/l and higher In an in vivo study Silverman and Silverman (22) obtained similar results Our study is in good accordance with that of MacGregor et al in higher concentrations However unlike MacGregor we also investigated concentrations below 1 g/l and found a statistically significant increase in adherence at 0 2 g/l as well as a tendency to increased adherence at *other low concentrations* Because of well known difficulties in counting leucocytes microscopically we used an automatic leucocyte counter A disadvantage with this is that granulocytes cannot be differentiated from other leucocytes but we found the automation superior from all other points of view

The effect of alcohol on leucocyte phagocytosis has not been investigated extensively Animal studies by Louria (10) suggested that the phagocytic function of polymorphonuclear leucocytes and their ability to kill infected material are impaired by alcohol In an in vitro study by Brayton et al (1) utilizing a modification of the Maaløe technique no impairment of phagocytosis and intracellular killing in leucocytes exposed to alcohol 2 or 4 g/l could be detected *Neither could any effect be detected* on leucocytes from healthy persons who had received alcohol infusion In our phagocytosis studies with the *oil emulsion* technique *we found a slight* tendency to improved phagocytosis at 0 04 g/l and a significant decrease of phagocytosis at 6 4 g/l and higher concentrations i e phagocytosis is only impaired at concentrations of alcohol which cannot be obtained clinically

Our phagocytosis and bactericidal studies utilizing the Maaløe technique which is less exact gave results similar to those from our experiments with the oil emulsion technique A tendency to decreased killing of bacteria is obtained at 6 4 g/l and a strikingly decreased killing is seen at alcohol 64 g/l

From a clinical point of view it is evident from our study that alcohol per se does not significantly influence chemotaxis adherence phagocytosis and intracellular killing negatively However an observation of great interest not reported before is the increased adherence phagocytosis and chemotaxis at low to moderate concentrations of alcohol

There still remain influences of alcohol per se for instance on glottis closure (17) and i v administration of alcohol decreases serum bactericidal activity (8) as well as total hemolytic complement (15) which are possible explanations for the presumed increased susceptibility to infections of chronic alcoholics Furthermore our study does not exclude the possibility that various problems associated with chronic alcoholism like malnutrition and hepatic cirrhosis (13) can influence the function of the inflammatory response

ACKNOWLEDGEMENT

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Abnormal Microheterogeneity of Transferrin in Serum and Cerebrospinal Fluid in Alcoholism

H Stibler C Allgulander S Borg and K G Kjellin

From the Department of Neurology and Karolinska Institute Clinical Department of Alcohol and Drug Research Karolinska Hospital Stockholm Sweden

ABSTRACT The serum and CSF proteins were analyzed by isoelectric focusing in 16 male alcoholics after alcohol intoxication and after 10–14 days of alcohol abstinence An abnormally marked protein band with pI 5 7 was found in serum in 15 patients and in CSF in 12 at the first examination On crossed immunoelectrofocusing it appeared as an increased cathodal, microheterogeneous molecular form of transferrin The abnormality was reversible and decreased or normalized in serum in all cases after abstinence In 6 patients with clinical signs of cerebellar degeneration an abnormal microheterogeneous pattern of CSF transferrin of partly different appearance to that in serum remained after abstinence Disturbed liver synthesis of transferrin is a probable origin of the serum finding which may be specifically related to alcohol abuse Substitution or loss of acidic amino acids and/or decreased iron binding ability are possible structural explanations

Quantitative serum protein alterations are well known in alcoholism especially with concomitant liver disease (1 5 9 17 35) In the work by Weeke (35) where quantitative rocket immunoelectrophoresis was used decreased levels of albumin haptoglobin and transferrin and increased values of orosomucoid α_1 antitrypsin α_2 macroglobulin haemopexin C 3 complement and immunoglobulins were common in alcoholic liver cirrhosis These findings were related partly to reduced liver protein synthesis and partly to an acute inflammatory reaction The cerebrospinal fluid (CSF) proteins have recently been studied by isoelectric focusing in 10 patients with cerebellar degeneration in association with alcoholism (32) In 8 of the cases 1–3 markedly increased protein bands were found between the main transferrin and the tau transferrin of CSF It was also observed that one of these bands (isoelectric point pH 5 7) was increased in serum in half of the patients On crossed immunoelectrofocusing these proteins appeared as increases of cathodic microheterogeneous molecular forms of transferrin (30) The CSF protein abnormalities were different from those previously described in hereditary cerebellar disorders (18)

The occurrence of a qualitative change in the microheterogeneity of a protein in CSF as well as in serum in a sequel to alcoholism made us ask whether this particular alteration was related to the cerebellar disease and/or to effects of alcohol outside the CNS The purpose of this investigation was to examine the serum and CSF proteins by isoelectric focusing even in alchoholics without cerebellar degeneration and to study the relation of the protein abnormalities to alcohol intake A preliminary report has been presented in abstract (31)

PATIENTS

The patients investigated were 16 males consecutively admitted in 1976 to the Karolinska Institute Clinical Department of Alcohol and Drug Research They were admitted due to sequels of alcohol abuse and did not suffer from other known diseases at the time of the investigation Nor had they experienced head trauma epileptic seizures or psychotic symptoms during the weeks preceding admission

The patients ages were between 26 and 63 years one half being 30–50 years old Alcohol abuse was estimated to have lasted for 5–15 years in seven cases and in four cases for less than 5 years According to medical records delirium tremens had previously occurred in three cases and epileptic seizures in two in association with alcohol abuse A concomitant abuse of diazepam was noted in one case

The majority of patients were employed while three were unemployed and two were receiving a disability pen



Fig 1 Isoelectric focusing of serum and CSF proteins (*A*) Normal serum to the left normal CSF from the same subject to the right The anode at the top and the cathode at the bottom The 10 numbered regions which are used to indicate the position of proteins are marked in the left margin (*s a* =point of sample application) The pH intervals of the numbered regions are given in Table I (*B*) Serum sample I (after alcohol intoxication) to the left and *CSF sample I to the right from the same patient without* cerebellar symptoms Note the marked bands in region *4 a* and *4 b* and the faint band in region 5 in serum and the parallel increase in the bands in region *4 a* and *4 b* in CSF (*C*) Serum sample II (after abstinence) to the left and CSF sample II to the right from the same patient as in Fig 1*B* The serum as well as the CSF protein pattern have now ..ized Abnormalities are indicated by arrows

sion Only one was homeless Four were married six single and six divorced

Pentobarbital was given on admission and during the first 4–7 days The initial dose was 600–1000 mg/day decreasing as abstinence symptoms subsided In one case oxazepam was given due to barbiturate allergy Three patients originally participating in the study dropped out before the second examination

METHODS

Clinical examinations All patients underwent a clinical neurological examination in connection with each sample collection Tremor of the legs was recorded in the sagittal and lateral planes on a statometer according to Silfverskiold (27)

CSF and serum samples were collected on two occasions on the first morning after admission (sample I) and after 10–14 days of alcohol abstinence (sample II) 10 ml of CSF were withdrawn by lumbar puncture performed in a standardized way (forenoon patient fasting)

Routine laboratory analyses Liver enzyme levels (ASAT ALAT LD and CK) bilirubin and alkaline phosphatase were determined routinely in both serum samples in all cases as was the serum iron content in 9 CSF was examined for cells and for total protein concentration by a modification of the method of Lowry et al (21)

Isoelectric focusing CSF and serum were analyzed by isoelectric focusing in thin layer polyacrylamide gel (18) For this purpose CSF was concentrated and serum was diluted 10 times The samples were stored for at most one week at +4 C before isoelectric focusing The Ampholine solutions (LKB) used were pH 3 5–10 (1 4 ml) pH 4–6 (0 1 ml) pH 5–7 (0 1 ml) and 9–11 (0 4 ml) pH measurements were made with a surface electrode (Type LOT 403 30-M8 Ingold Zurich Switzerland) at +20°C (33) The controls consisted of 25 subjects with minor psychic disturbances and 32 healthy volunteers in the age range 19–57 years (approved by the Ethical Committee of the Karolinska Institute Stockholm)

For practical purposes the gel has been divided into 10 numbered regions (19) to indicate the position of proteins (Fig 1*A*) The divisions are based on the positions of the normal protein bands In CSF region 1 includes up to 9 bands e g orosomucoid haptoglobin α_1 antitrypsin ceruloplasmin and prealbumin region 2 corresponds mainly to albumin and region 3 contains up to 5 bands e g the main fractions of α_2-macroglobulin transferrin and C 3 complement Region 4 includes in CSF 3–6 bands corresponding to parts of transferrin α_2 macroglobulin C 3 complement and to haemopexin while region 5 consists of the sialic acid deficient tau transferrin fraction of CSF (23 24) and parts of α_2 macroglobulin and C 3 complement (30) No distinct bands are usually found in region 6 in CSF Regions 7–9 indicate the normal gammaglobulin area and region 10 corresponds to the most alkaline pH range where no distinct bands are normally visible The pH intervals of these numbered regions are shown in Table I (32)

Crossed immunoelectrofocusing and electroimmunoassay Two-dimensional crossed immunoelectrofocusing of

Table I The pH intervals corresponding to the numbered regions of isoelectric focusing

The pH values are mean values from measurements on five gels s a =sample application (32)

Region	pH interval
1	2 5–4 7
2	4 7–5 0
3	5 0–5 4
4	5 4–5 8
5	5 8–6 0
6	6 0–6 2
s a	6 2–6 4
7	6 4–7 4
8	7 4–8 2
9	8 2–8 9
10	8 9–11 0

Table II *Results of isoelectric focusing of serum and CSF proteins in 16 patients with alcoholism*

Sample I=examinations on admission sample II=examinations after 10–14 days of abstinence CSF sample II shows the number of patients with abnormalities which seemed not to be explained by the influence of remaining serum protein alteration The pH regions are defined in Fig 2A and Table I Figures in parentheses denote no of patients with remaining but reduced abnormality HAF=highly alkaline fraction (pI 9 3)

	No of pats with protein abnormality in the following pH regions						
	4 a	4 b	4 c	4–5	5	7	10 (HAF)
Serum							
Sample I	8	15	1	0	4	0	0
Sample II	0 (3)	0 (7)	0	0	0 (2)	0	0
CSF							
Sample I	9	12	3	4	6	1	0
Sample II	6	8	2	4	6	1	2

Double fractions only present in CSF

6 of the CSF and 7 of the serum samples was carried out according to Soderholm and Smyth (29) and Stibler (30) Rabbit antisera against human α_2 macroglobulin transferrin and C 3 complement were purchased from Dakopatts Denmark and against haemopexin from Behringwerke West-Germany In addition both serum samples from 8 patients were examined for total transferrin concentration by single rocket immunoelectrophoresis (20)

To study a possible change in sialic acid content of abnormal protein bands patient sera and normal sera were treated with neuraminidase (from Vibrio cholerae Behringwerke West-Germany containing 500 U/ml) The sera were incubated with increasing amounts of enzyme corresponding to 0 2 0 5 1 0 2 0 4 0 8 0 and 40 0 μl neuraminidase/15 μg of transferrin at 37°C for 24 hours (22 23) and analyzed by isoelectric focusing and crossed immunoelectrofocusing

The influence of the iron content on the pI of transferrin was examined by isoelectric focusing Purified human apotransferrin (Behringwerke West-Germany maximal iron content 20 μg/g apotransferrin) was incubated with $FeCl_3$ at 37°C for 1 hour to give 0 3 mg iron/g transferrin (23% saturation) and 5 9 mg iron/g transferrin (>100% saturation)

RESULTS

Clinical findings The clinical examination including the recordings of tremor of the legs showed in 6 patients signs of the cerebellar syndrome occurring in chronic alcoholism (2 4 25 34) The tremor of the legs was slow and coarse with a frequency of 3 cps especially in the lateral statometer recordings as described by Silfverskiold (27) Four of these cases also exhibited a clinically demonstrable polyneuropathy usually of slight degree of the distal parts of the lower extremities This latter finding was also made in 2 other patients The clinical signs were unchanged between the first and second examinations

Routine laboratory findings The CSF cell count was normal in all cases The CSF total protein concentration was slightly or moderately increased in sample I in 13 patients (0 56–0 79 g/l normal range 0 20–0 50) in sample II it had decreased or normalized in 8 instances In the patients with cerebellar degeneration the protein concentration remained increased in sample II except in one case

The liver enzyme levels were increased in all patients except one and the bilirubin concentration was increased in one case in serum sample I In sample II the enzyme activities were decreased or normalized in all patients In no case were there any clinical signs of hepatic insufficiency The serum iron content was at the upper normal level or slightly increased in 7 of the 9 cases in sample I and decreased usually to subnormal values in sample II

Isoelectric focusing The results of isoelectric focusing of the serum and CSF proteins are shown in Table II and Figs 1 2 and 3 The most common abnormality was a marked increase in the band in region 4 b (Fig 1*A*) with an isoelectric point of 5 7 in serum as well as in CSF at the first examination (Fig 1*B*) It was absent in serum only in one patient who had the least serious alcohol abuse Increases in other serum proteins in the pH 5 interval (region 4 a pI 5 6 region 4 c pI 5 8 and region 5 pI 5 9) were less frequently found In sample II after 10–14 days of alcohol abstinence the serum altera

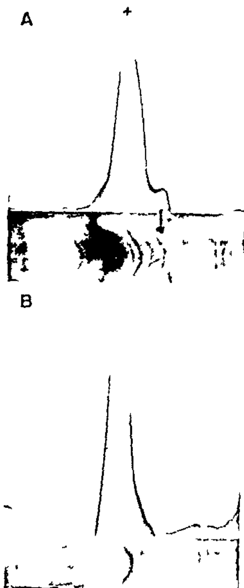


Fig 2 Crossed immunoelectrofocusing of serum proteins Reference gel strips from the first dimension isoelectric focusing are shown at the bottom with the anode to the left and the cathode to the right Only regions 1–6 (Fig 1A) are included In the second dimension immunoelectrophoresis the anode was at the top Abnormalities are indicated by arrows (*A*) Serum sample I (after alcohol intoxication) examined with antibodies against human transferrin Note the marked microheterogeneous immunoprecipitate cathodal to the main peak corresponding to the abnormal band in region *4b* (Fig 1*A* and *B*) on isoelectric focusing (*B*) Serum sample II (after abstinence) examined with antibodies against human transferrin The main peak has increased and the microheterogeneity is now of normal appearance

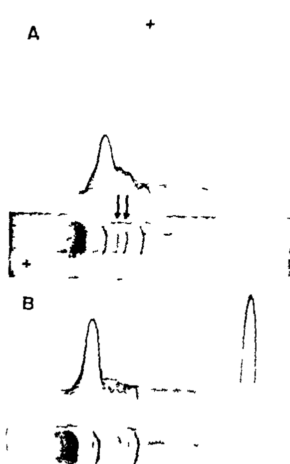


Fig 3 Crossed immunoelectrofocusing of CSF proteins using antibodies against human transferrin Reference gel strips from the first dimension isoelectric focusing are shown at the bottom with the anode to the left and the cathode to the right All regions 1–10 are included In the second dimension immunoelectrophoresis the anode was at the top To the right are reference transferrin samples Abnormalities are indicated by arrows (*A*) CSF (after abstinence) from a patient with alcoholic cerebellar degeneration Note the two markedly increased microheterogeneous immunoprecipitates cathodal to the main peak corresponding to the abnormal bands in region *4a* and *4b* (Fig 1*A*) on isoelectric focusing (*B*) Normal CSF transferrin pattern (30)

tions were clearly reduced or had normalized in all cases (Fig 1*C*) Serum from 2 patients was also examined at daily intervals for the first 5 days The increased band in region 4b was unchanged in both cases in all 5 samples In the CSF increased or abnormal bands remained in sample II in 9 patients which seemed not to be explained by the influence of remaining serum protein abnormalities All of the 6 patients with signs of cerebellar degeneration

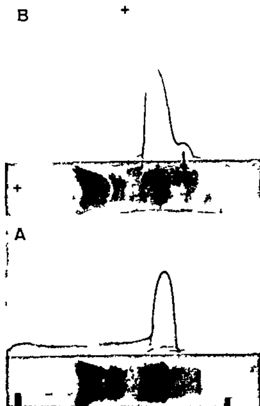


Fig 4 Crossed immunoelectrofocusing of serum proteins Reference gel strips from the first dimension isoelectric focusing are shown at the bottom with the anode to the left Only regions 1–7 (Fig 1*A*) are included In the second dimension immunoelectrophoresis the anode was at the top Abnormalities are indicated by arrows (*A*) Normal serum examined with antibodies against human transferrin after total desialylation with neuraminidase The pI of transferrin has increased to 5 9 (*B*) Serum sample I (after alcohol intoxication) examined with antibodies against human transferrin after total desialylation One additional immunoprecipitate is found with pI 6 2

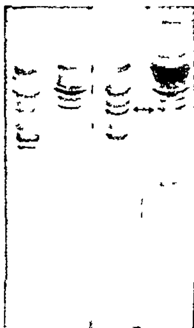


Fig 5 Isoelectric focusing from left to right of human apotransferrin (Behringwerke West-Germany maximal iron content 20 μg/g apotransferrin) fully iron saturated transferrin transferrin containing 0 3 mg iron/g transferrin and serum sample I from a patient after alcohol intoxication The anode was at the top The arrow indicates the probable position of Tf Fe 1 and the abnormal transferrin band in the patient s serum both with the pI of 5 7

showed remaining increases in 1–3 of the bands in region 4 and extra fractions (regions 4–5 5 7 and 10) were noted in a few cases These findings are in accordance with those previously reported (32) In 3 patients without detectable cerebellar degeneration CSF protein abnormalities were still observed in sample II One of these subjects suffered from a polyneuropathy and showed an abnormal statometer test though not with the typical tremor of 3 cps In the other 2 cases no obvious clinical explanation could be found In patients with increased CSF protein concentration slight or moderate signs of blood–CSF barrier damage were observed (19) No protein changes of the kind described were found in sera from a limited number of patients with non alcoholic liver disease or with anaemias The serum as well as the CSF protein alterations were stable at −23 C for at least 6 months

Crossed immunoelectrofocusing and electroimmunoassay On two-dimensional crossed immunoelectrofocusing using antiserum against transferrin the band in region 4b (Fig 2*A*) and the one in region 5 in serum appeared as markedly increased microheterogeneous forms of transferrin with more alkaline isoelectric pHs than the main fraction of transferrin (pI 5 7 5 9 and 5 4 respectively) Normally in region 5 there is not any visible immunoprecipitate of transferrin in serum These changes were reduced or normalized in sample II (Fig 2*B*) On the other hand the main precipitate of transferrin was generally lower in sample I than in sample II The quantitative measurements of

transferrin in serum using single rocket immunoelectrophoresis also showed that the total quantity was lower in sample I than in sample II (difference 0 1–1 1 g/l average 0 4 g/l) but still within the normal range The change in the microheterogeneous *pattern of transferrin in serum was therefore qual*itative but reversible In CSF the bands in region 4a and 4b also corresponded to increased microheterogeneous transferrin forms which decreased or returned to normal in sample II in all but 2 patients without cerebellar symptoms In patients with signs of cerebellar degeneration however they remained increased (Fig 3)

When α_2 macroglobulin was examined with the same technique a general increase in the 3 main microheterogeneous forms was found in serum sample I One of these forms was at least partly responsible for the increased band in region 4a (pI 5 6) No change in the α_2-macroglobulin pattern was found between CSF samples I and II although it *appeared slightly increased in both samples* Considering its very low total amount in CSF this protein is probably not responsible for any of the observed abnormalities (3)

Crossed immunoelectrofocusing of serum with antiserum against C'3 complement showed a marked increase in the main anodal immunoprecipitate in sample I which clearly decreased in sample II Its position is however too anodal to be responsible for any of the described abnormalities The single homogeneous immunoprecipitate of haemopexin in serum (pI 5 7) was of normal appearance

Treatment of purified human iron saturated transferrin with neuraminidase results in a stepwise *cathodal displacement of its position on electro*phoresis (22 23) On isoelectric focusing the main native band with pI 5 4 dissolves into 4 successive bands with pI 5 6 5 7 5 8 and 5 9 After total desialylation all transferrin is found as one band with pI 5 9 (Stibler to be published) Neuraminidase treatment of whole serum resulted in the same successive cathodal displacement of transferrin After total desialylation of normal serum (when further increase in enzyme concentration or incubation time gave no additional change in the protein pattern) all transferrin was found as one band with pI 5 9 which gave one single immunoprecipitate on crossed immunoelectrofocusing (Fig 4*A*) In patient sera however one additional transferrin band was found 0 3 *pH units (pI 6 2)* cathodal to the normal asialotransferrin and gave an abnormal transferrin precipitate on crossed immunoelectrofocusing (Fig 4*B*) This is the same pI difference as between the native main band of transferrin (pI 5 4) and the abnormal band in region 4b (pI 5 7) in *patient sera This finding* speaks against any difference in sialic acid content between the transferrin in region 4b and the normal protein (22 23 24) If it was due to different sialic acid amounts the difference would have disappeared after total desialylation and one band and one immunoprecipitate would have been found

The pI of the main band of human apotransferrin was found to be 6 0 After saturation with iron the pI of the main band decreased to 5 4 Partial saturation with 0 3 mg iron/g transferrin gave both these bands but also a marked intermediate band with pI 5 7 (Fig 5) This latter band corresponds to the pI found by Hovanessian and Awdeh (15) for transferrin with one bound iron atom (Tf-Fe 1)

DISCUSSION

Isoelectric focusing combined with immunoelectrophoresis offers a new possibility to study qualitative changes in protein microheterogeneity (10 16 26 28 29 30)

In this investigation a qualitative alteration of the transferrin pattern in serum was found in almost all cases after alcohol intoxication A similar parallel finding was made in CSF It decreased or normalized in serum in all patients after 10–14 days of alcohol abstinence (Fig 1) The abnormality consists of an increased microheterogeneous form of transferrin with a more alkaline isoelectric point (pI *5 7) than the normal main fraction of this protein* (pI 5 4) (Fig 2*A* and *B*)

Several factors may be responsible for such an alteration of transferrin Transferrin with only one bound iron atom (Tf Fe 1) to one of its two iron binding sites has an isoelectric point of 5 7 on thin layer isoelectric focusing (15) (Fig 5) It is known that the serum iron level increases in alcoholism (13) In the present cases the serum iron value was at the upper level of normal or only slightly increased in 7 of 9 examined patients If the transferrin change was caused simply by an increased iron level transferrin with two bound iron atoms (Tf Fe 2 pI 5 4) would also be increased since the relation Tf Fe 2 Tf Fe 1 is 10 1 in serum according to Hovanessian and Awdeh (15) On the contrary

this fraction as well as the total transferrin con centration was generally lower in sample I (see Methods) than in sample II Incubation of patient sera with $FeCl_3$ in excess and of normal sera with varying amounts of $FeCl_3$ as well as with ethanol could not abolish or reproduce respectively the transferrin abnormality Ethanol given to three healthy volunteers (125 g in one dose) caused a rapid change in the serum iron level but did not give the transferrin alteration If this change in trans ferrin is related to increased Tf Fe 1 then the iron binding ability would be qualitatively decreased in alcoholism

A decreased sialic acid content of transferrin also displaces its pI cathodically which however is contradicted in this case by the result of neur aminidase treatment (Fig 4) Substitution or loss of acidic amino acids are other possible explanations alone or combined with altered iron binding ability The half life of transferrin is 6–12 days (8) and it takes 6–10 days of alcohol abstinence before any decrease in the abnormality is observed Interfer ence with the liver synthesis of transferrin by pro-longed alcohol intake is therefore a probable mechanism behind the abnormal serum transferrin pattern This alteration may be specific for alcohol abuse since it was not found in sera from the pa tients with non alcoholic liver diseases so far ex amined

The abnormal microheterogeneity of CSF trans ferrin in patients with cerebellar degeneration (30 31) (Fig 3) seems to be the result of some other mechanism as well since the findings are of partly different appearance to those in serum and remain after abstinence when the influence of the serum protein alterations would have ceased Remaining CSF protein abnormalities in 3 of the present pa tients without clinically detectable cerebellopathy may indicate subclinical cerebral and/or cerebellar lesion

The abnormal microheterogeneity of serum transferrin is now subject to further studies espe cially the possible qualitative disturbance in iron binding ability The affinity to cell receptors is dif ferent for human Tf Fe 1 and Tf Fe 2 and the two iron binding sites are considered to have different specificity for various cells (6 7 11 12) A de creased capacity for transferrin to bind two iron atoms may therefore be of importance with respect to sequels to alcoholism e g haematological (13 14) and neurological complications

ACKNOWLEDGEMENT

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Does a Disturbed Insulin Release Promote Hypoglycemia in Alcoholics?

Ragnar Hed, Arne Nygren, Sven Rojdmark and Lars Sundblad

From the Departments of Internal Medicine II and Clinical Chemistry, Sodersjukhuset, Stockholm, Sweden

ABSTRACT Peroral glucose tolerance tests, i v tolbutamide tests and postfasting alcohol tests were performed in three alcoholics who had been admitted to hospital with hypoglycemic symptoms. In one case the findings indicated extreme insulin sensitivity and in two cases there was evidence of a disturbed insulin release. These two factors might have a bearing on the occurrence of hypoglycemic symptoms in alcoholics.

It is well known that in fasting man alcohol ingestion may result in hypoglycemia. This effect of alcohol is considered to be due to an inhibition of gluconeogenesis at a time when the hepatic glycogen stores are depleted (2, 3). Malnutrition and drinking in the fasting state is a very common combination in chronic alcoholics. However, alcoholics seldom develop hypoglycemic coma (5). Probably alcoholics are hypoglycemic many times without any dramatic symptoms. Perhaps symptoms related to alcohol hypoglycemia might occur only in subjects with an unusual susceptibility. Such a concept is supported by the findings in the present report on three alcoholics who had displayed hypoglycemic episodes.

METHODS

All tests were performed in the morning. Blood samples for glucose and immunoreactive insulin (IRI) determinations were drawn from an antecubital vein. Blood glucose was determined enzymatically with a commercial glucose oxidase test kit (Kabi reagents, Sweden). Serum IRI was assayed by a double antibody procedure essentially as described by Soeldner and Slone (7).

Oral glucose tolerance test. Glucose (100 g) was given after an overnight fast (12–14 hours). The glucose was dissolved in 400 ml water and ingested by the patient over a period of not more than 5 min. A normal oral glucose tolerance test was defined according to the criteria given by Joplin and Wright (4), i e a 1 hour value less than 9.0 mmol/l and a 2 hour value less than 6.7 mmol/l. In our laboratory the following IRI values (M + S D) were found in 11 healthy non-obese subjects (20–51 years old) with normal glucose tolerance

Min	IRI (mU/l)	Min	IRI (mU/l)
0	15+10	120	63+29
30	81+35	180	40+24
60	89+34		

Tolbutamide test. After an overnight fast (12–14 hours) 1 g of tolbutamide was injected into an antecubital vein over a period of 2 min. The mid point of the injection was taken as zero time. In 11 healthy non-obese subjects (20–51 years old) with normal oral glucose tolerance the following glucose and IRI values were found (M + S D)

Min	Glucose (mmol/l)	IRI (mU/l)
0	4 1+0 4	17+ 8
2		104+62
10	3 3+0 3	67+29
60	2 2+0 4	21+13
120	3 1+0 5	
180	3 6+0 4	16+ 8

Postfasting alcohol test. After 44 hours of fasting the patient was given 40 ml of 96% alcohol (vol/vol) diluted with water to 15–30% alcohol. In 7 non-obese healthy subjects (22–30 years old) with normal glucose tolerance the following glucose and IRI values (M + S D) were found

Min after alcohol intake	Glucose (mmol/l)	IRI (mU/l)
0	3 1+0 5	6+2
30	2 6+0 5	3+1 5
60	2 4+0 6	3+1.5
90	2 3+0 5	4+2
120	2 1+0 3	3+1
150	2 0+0 3	4+1 5
180	2 1+0 4	4+1 5
210	2 2+0 4	4+2
240	2 3+0 4	4+1

STUDY BASE

The patients were non-obese men who had abused alcohol for many years, consuming large quantities daily for periods ranging from 3–4 weeks up to 6 months. Such an alcohol bout had preceded the present admissions to hospital. The Hb concentration, WBC and ESR were all normal, as were serum sodium, potassium, standard bicarbonate and creatinine. They were given a normal hospital diet (about 250 g carbohydrate per day) for at least three

Table I *Results of liver examinations performed during the first hospital stay*

Case no	Bilirubin (µmol/l)	Prothrombin index (%)	Alkaline phosphatases (µkat/l)	ASAT (µkat/l)	ALAT (µkat/l)	Liver biopsy
1	10	80	2 1	1 1	0 9	-
2	10	100	1 5	0 4	0 2	Slight steatosis
3	12	80	3 2	1 3	0 8	Slight steatosis
Normal range	4–21	70–130	1 0–3 5	<0 7	<0 7	

days before the glucose tolerance and tolbutamide tests Results of liver tests are given in Table I

Case 1 (Table II)

This patient (26 years old) was admitted to a psychiatric clinic after an alcohol period During the first week in hospital he felt dizzy every morning and appeared somnolent recovering after breakfast In that week fasting glucose varied between 0 8 and 1 7 mmol/l Thereafter fasting glucose was normal and he felt well Physical examination was unrevealing He returned to the hospital one year later after a new alcohol period This time no symptoms indicating hypoglycemia were observed and fasting glucose varied between 2 7 and 5 5 mmol/l

Case 2 (Table III)

This man (41 years old) arrived comatose at the emergency ward Blood glucose was 1 4 mmol/l He was given hypertonic glucose i v and recovered During the following days in hospital he felt well and no spontaneous hypoglycemia occurred Physical examination was unrevealing

Case 3 (Table IV)

This man (58 years old) was brought to the emergency ward of the hospital in a comatose state Blood glucose was 1 7 mmol/l He was given hypertonic glucose i v and *regained consciousness Two hours later he became* drowsy and at this moment blood glucose was 1 8 mmol/l Again hypertonic glucose was given i v with prompt effect During the following 24 hours he was found in a somnolent state four more times despite a continuous i v infusion of isotonic glucose Altogether 250 ml of 30% glucose was given i v during that day and night During the second day he felt well In the afternoon of the third day in hospital he suddenly became disordered and blood glucose was low once more (1 4 mmol/l) This time too an i v injection of hypertonic glucose had to be given After that there were no hypoglycemic episodes

During the next seven years he was readmitted several times for observation He never again experienced any hypoglycemic symptoms and hypoglycemia did not occur when he fasted for two days Physical examination never revealed any signs indicating liver disease

Table II *Glucose (mmol/l) and immunoreactive insulin (IRI) (mU/l) during various tests in case 1*

Mo after first admission										
	Peroral glucose tolerance test									
	Minutes	*0*	*30*	*60*	*90*	*120*	*150*	*180*		
0	Glucose	2 8	7 9	9 1	5 1	4 8	4 4	3 4		
	IRI	0–1	14	8	8	6	0–2	0–1		
10	Glucose	2 8	5 9	8 3	9 0	8 8	6 9	5 2		
	IRI	10	75	76	64	53	58	39		
	I v tolbutamide test									
	Minutes	0	2	10	20	30	60	90	120	180
0	Glucose	2 8		2 7	1 0	1 1	0 7	0 8	1 4	1 7
	IRI	0–1	8	0–2	0–1	0–1			0–1	
10	Glucose	2 7		2 0	2 0	1 7	1 1	1 6	1 9	2 2
	IRI	22	84	80		22	16			18
	Postfasting alcohol test									
	Minutes	0	30	60						
0	Glucose	1 9	1 2	0 7*						
	IRI	4	0	2						

* At this time the patient became drowsy and an i v injection of hypertonic glucose had to be given

Table III *Glucose (mmol/l) and immunoreactive insulin (IRI) (mU/l) during various tests in case 2*

Peroral glucose tolerance test									
Minutes	0	30	60	90	120	150	180		
Glucose	2 9	7 6	7 8	7 7	4 4	2 6	2 3		
IRI	0	274	200		40		0		
I v tolbutamide test									
Minutes	0	2	10	20	30	60	90	120	180
Glucose	3 4		3 2	3 1	2 0	1 8	1 7	2 4	2 8
IRI	4	2	20			10		8	
Postfasting alcohol test									
Minutes	0	30	60	90	125	150	180	210	
Glucose	2 2	1 9	1 6	1 3	1 1	1 0	1 1	1 2	
IRI	4	12	4	4	4	12	2	4	

RESULTS AND DISCUSSION

These three alcoholics demonstrate two factors which might have a bearing on the occurrence of hypoglycemic symptoms in alcoholics

Extreme insulin sensitivity

Case 1 showed when first examined nearly unmeasurable fasting IRI values Following glucose ingestion (100 g) IRI increased to 14 mU/l and with this small amount of insulin he maintained a normal glucose tolerance At the tolbutamide test IRI increased to 8 mU/l resulting in a profound hypoglycemia (0 7 mmol/l) When challenged with alcohol after fasting for 44 hours blood glucose decreased to 0 7 mmol/l and he thereby became drowsy Probably the morning hypoglycemia and the insulin sensitivity were related to a decreased gluconeogenesis It is well known that the hepatic gluconeogenesis is reduced during alcohol combustion (2 3) Moreover it is possible that certain hormonal disturbances found in alcoholics might contribute to a decrease in gluconeogenetic capacity Thus subnormal responses in plasma cortisol have been found in some alcoholics when their

Table IV *Glucose (mmol/l) and immunoreactive insulin (IRI) (mU/l) during various tests in case 3*

Years after first admission										
	Peroral glucose tolerance tests									
	Minutes	0	30	60	90	120	150	180		
4	Glucose	5 0	8 1	8 4	9 8	8 4	8 7	7 5		
	IRI	78	540	416		400		350		
5	Glucose	4 4	4 8	3 1	2 9	4 8	5 1	5 1		
	IRI	29	311	189	48	135	135			
7	Glucose	5 1	7 2	7 5	6 0	6 5		5 9		
	IRI	2	116	122	46	74		76		
	I v tolbutamide tests									
	Minutes	0	2	10	20	30	60	90	120	180
4	Glucose	4 4		3 8	2 5	1 7	1 9	3 1	3 6	4 0
	IRI	124	944	728	428	180	92	70	100	86
5	Glucose	3 8		2 6	1 3	0 7	1 2	1 9	2 6	2 5
	IRI	20	470	318			96			
	Postfasting alcohol tests									
	Minutes	0	30	60	90	120	150	180	210	240
4	Glucose	3 3	3 0	2 7	1 5	2 0	2 5	2 6	2 8	3 0
	IRI	44	32	24	22	22	22	16	26	16

adrenocortical function was stimulated by i m injection of a synthetic ACTH preparation (Synachten®) (6) Other findings have been reported which point to an insufficient ACTH release in some alcoholics during hypoglycemia (8) Possibly a state of incomplete and transient adrenocortical insufficiency might occur in alcoholics contributing to a decrease in gluconeogenesis which might persist several days after the last alcohol intake When case 1 was readmitted one year later he did not display any hypoglycemic symptoms On this occasion he was not particularly sensitive to the effect of insulin either

Disturbance of the insulin release mechanism

In case 2 a peroral glucose intake elicited a rapid rise of IRI to 274 mU/l which seems rather high in relation to the insulin sensitivity displayed after the tolbutamide injection Hypoglycemia was observed at the end of the glucose tolerance test and occurred also during a postfasting alcohol test but this was accompanied by an increase in IRI above the pretest value

Case 3 showed when examined 4 years after the first admission a slightly decreased glucose tolerance Fasting IRI was high (78 mU/l) and after the oral glucose intake the IRI level increased to 540 mU/l A tolbutamide test showed an excessive insulin response (peak IRI value 944 mU/l) and a normal fall of blood glucose When alcohol was ingested ter a 44 hour fast blood glucose decreased to ypoglycemia IRI levels varied between 16 and 32 U/l indicating that insulin was continuously released in spite of the hypoglycemia One year later (i e 5 years after the first admission) oral intake of 100 g glucose resulted in a restricted increase in blood glucose The peak IRI value was 311 mU/l At the same time a tolbutamide test showed prolonged hypoglycemia The maximal IRI increase was 470 mU/l During the seven years of observation after the first admission case 3 never again displayed hypoglycemic symptoms Hypoglycemia did not occur when on several occasions the patient fasted for two days in hospital A peroral glucose tolerance test performed seven years after the first admission was normal It is evident that case 3, when examined 4 years after the first admission needed large amounts of insulin to maintain a normal glucose homeostasis When this condition of insulin resistance was on the verge of disappearing the hypersecretory capacity of the β cells probably persisted Glucose and tolbutamide stimulation accordingly resulted in an insulin release which was inappropriately large The results of these two tests were at that time compatible with the diagnosis of insuloma It is evident that these insuloma like tests reflect a transitory state of hypersecretion by the β cells Perhaps the regulation of insulin release was also disturbed during the first hospital stay in this case A release of insulin in excess of the requirement after glucose administration would explain the recurrence of hypoglycemia several times during the first days of that hospital stay

During alcohol hypoglycemia blood insulin decreases and this decline appears to represent an important counter regulatory mechanism to protect against the devasting effect of hypoglycemia on the central nervous system (1) Accordingly clinical hypoglycemia might ensue if insulin release continues in spite of a low blood glucose Perhaps such a mechanism explains the clinical hypoglycemia in cases 2 and 3 The insulin sensitivity found in case 1 probably reflects a persistent impairment of gluconeogenesis It is possible that this defect and the disturbed insulin release found in cases 2 and 3 are related to their alcohol abuse Anyhow it is likely that an impairment of gluconeogenesis combined with a disturbance of insulin secretion would render an alcoholic extremely vulnerable to drinking in the fasting state

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Exercise Performance and Body Dimensions in Anorexia Nervosa before and after Rehabilitation

Lars Fohlin

From the Department of Pediatrics Karolinska Institutet St Goran s Children s Hospital Stockholm Sweden

ABSTRACT Three boys and five girls (mean age 15 0 y) with anorexia nervosa (AN) were studied before and after a treatment which restored their body weight to normal Before treatment the patients average weight loss was 25% of their pre morbid weight The function and dimensions of the oxygen transport system were determined with heart (HV) and blood (BV) volumes, lean body mass (LBM) and exercise tests on a bicycle ergometer with determination of maximal aerobic power (V_{O_2} max) Before treatment the patients had bradycardia and hypotension HV and BV decreased in proportion to the loss of body weight During maximal exercise, attainable oxygen uptake and heart rate were low V_{O_2} max decreased out of proportion to the circulatory and body dimensions After treatment HV and BV increased in proportion to the rise in body weight LBM increased significantly in all patients Heart rates at rest and during exercise were within the range of normal and V_{O_2} max increased It is concluded that the circulatory system is highly adaptive to the low caloric intake in AN and is totally normalized after weight gain

Although most of the common clinical symptoms of anorexia nervosa (AN) such as bradycardia low BP low body temperature amenorrhea and lanugo hair are known to occur during long standing starvation (16) there is still no agreement about the pathogenesis and pathophysiology of the disease The endocrinological aspects of AN have attracted much scientific attention (12 14) and the possibility of a primary hypothalamic dysfunction has been discussed (18)

The biology of human starvation is well known (16) but there are few studies regarding the effects of undernutrition in AN on body structure and cardiovascular function Considering the reported increase in frequency (10) and the high mortality rate (5–10%) of the disease (20) there is a surprising lack of information concerning its pathophysiological aspects In previous studies the body composition (8) and cardiovascular (11) and renal function (1) in the pubescent anorexic child were investigated The present study was undertaken to evaluate the influence of rehabilitation therapy resulting in the restitution of normal body weight on body and circulatory dimensions and maximal aerobic power (V_{O_2} max) in eight young AN patients

PATIENTS AND METHODS

Eight patients three boys and five girls with AN were studied before and after weight gain They have all been included in previous investigations (1 8 11) They were studied after informed consent had been given by the Ethical Committee of Karolinska Institutet None of them had a history of overweight before the onset of AN and the average duration of weight loss was 1 0 year The mean age at first examination was 17 1 years for the boys and 13 8 years for the girls Their physical characteristics are shown in Table I The boys were postpubertal at the onset of the disease Two of the girls had secondary amenorrhea and the other three developed symptoms before the onset of menarche The interval to recovery varied between 0 4 and 2 9 years All the patients were treated in hospital for some time and were regularly seen by a psychiatrist None was on drug therapy When rehabilitated the patients with the exception of patient 8 returned to their premorbid weight They were reinvestigated immediately following rehabilitation except for one boy (patient 1) who had been rehabilitated for about 1 5 years and had trained extremely hard with long-distance running during that time Two girls (patients 4 and 5) gained their weights within 6 months

Blood volume (BV) was determined with ^{125}I labelled albumin (22) *Heart volume* (HV) was measured in the prone position (17) Skinfold thickness at triceps and subscapular sites was measured with a Harpenden caliper

Table I *Physical characteristics of three boys and five girls with anorexia nervosa before (I) and after weight gain (II)*

Pat no	Age (y) I	Age (y) II	Premorbid weight (kg)	Weight loss (%)	Weight (kg) I	Weight (kg) II	Height (cm) I	Height (cm) II
Male								
1	16 0	18 9	72	22	56 7	76 0	188	188
2	18 2	19 3	62	22	48 7	63 5	186	186
3	17 1	17 8	62	22	51 0	62 5	184	184
Female								
4	12 1	14 3	35	15	30 0	45 0	153	154
5	12 8	13 2	45	35	29 3	47 0	160	160
6	14 0	15 0	48	27	35 0	49 0	164	164
7	14 2	16 0	41	15	34 5	43 3	157	157
8	15 9	17 8	54	34	35 8	49 3	164	164
Mean	15 0	16 5	52 4	24	40 1	54 5	170	170
±S D	2 1	2 2	12 4	8	10 4	11 6	14	14
p	<0 01				<0 001		n s	

n s =Not significant

Table II *Triceps subscapular skinfold thickness and lean body mass in two boys and five girls with anorexia nervosa before (I) and after weight gain (II)*

Pat no	Triceps (mm) I	Triceps (mm) II	Subscapular (mm) I	Subscapular (mm) II	Lean body mass (kg) I	Lean body mass (kg) II
Male						
2	3 4	5 0	5 2	8 4	46	54 9
3	4 4	6 8	4 6	7 8	48 2	53 8
e						
4	3 4	13 8	3 8	12 6	29 2	33 6
5	2 6	7 0	2 8	9 0	29 3	39 0
6	5 0	11 0	4 4	7 4	31 5	39 9
7	4 6	12 8	4 6	8 4	31 4	35 9
8	6 2	11 0	5 0	12 4	32 6	39 7
Mean	4 4	9 6	4 3	8 0	35 5	42 4
±S D	1 4	3 4	0 8	3 0	8 1	8 5
p	<0 01		<0 01		<0 001	

From the sum of these *lean body mass* (LBM) was calculated (19) *Blood lactate* was determined by an enzymatic method (5) Detailed information regarding the methods is given elsewhere (8 11) Exercise was performed with the patients sitting on an electrically braked bicycle ergometer (*Siemens Elema*) with stepwise increasing work loads The criteria for maximal exercise were that blood lactate and respiratory quotient should exceed values of 9 mmol/l and 1 0 respectively (2) Two girls failed to fulfil these criteria The statistical significance of the observed values before and after rehabilitation was tested using Student s paired *t* test

RESULTS

Basic data concerning the patients at rest before and after rehabilitation are given in Tables II and III Their weight loss was 25% The boys had 5–9% of their total body weight as fat before treatment and 12–14% after weight gain when calculated from skinfold measurements Corresponding mean values for the girls were 6 and 20% Skinfold thickness increased significantly, which is well illustrated in Fig 1 LBM increased also significantly in all patients (mean 35 5 kg at the first and 42 2 kg at the second investigation) Their height remained unchanged between the two observations HV and BV increased significantly in all patients following weight gain (Figs 2 and 3) but the increase was on the whole proportional to the gain in weight Mean values for BV/kg b wt before and after treatment were 77 and 79 ml respectively At rest heart rate *was significantly faster ($p<0\ 01$) when the patients* were rehabilitated with a mean value of 79 beats/min against 53 beats/min before treatment Blood lactate at rest was not significantly changed after rehabilitation The mean oxygen uptake at rest increased from 0 015 to 0 020 l/min after weight gain

The responses to maximal exercise performance are shown in Table IV The maximal exercise heart rate increased significantly from 177 to 195 beats/min ($p<0\ 01$) and the absolute V_{O_2} max rose from 1 49±0 32 to 2 64±0 87 l/min ($p<0\ 50$) after weight gain *Expressed in terms of body weight* the corresponding mean figures were 36 and 46 ml/kg min

Table III *Resting circulatory data and dimensions before (I) and after treatment (II)*

Pat no	Heart rate (beats/min) I	Heart rate (beats/min) II	BP (mmHg) I	BP (mmHg) II	Heart volume (ml) I	Heart volume (ml) II	Blood volume (l) I	Blood volume (l) II	Blood lactate (mmol/l) I	Blood lactate (mmol/l) II
1	38	78	110/80	140/60	769	1 123	4 94	6 46	1 4	2 0
2	63	80	110/65	115/70	545	682	2 80	4 10	3 9	1 7
3	60	76	95/60	120/70	620	756	3 51	5 30	1 8	1 0
4	47	84	90/60	105/60	362	479	2 37	3 27	2 0	1 0
5	57	105	80/60	115/70	391	541	3 00	3 80	1 2	1 4
6	53	84	90/60	110/70	332	448	2 46	3 76	1 4	–
7	53	57	105/80	105/70	406	499	2 90	3 46	1 7	1 8
8	52	68	95/65	100/80	453	521	2 40	4 40	1 7	0 9
Mean	53	79	97/65	114/71	485	631	3 05	4 32	1 9	1 4
±S D	8	14	97±11 65±7[b]	114±12 70±8[b]	150	225	0 86	1 07	0 9	0 4
p	<0 01		<0 05[a] n s[b]		<0 01		<0 01		n s	

n s = Not significant
[a] Systolic [b] diastolic

Maximal blood lactate was not significantly changed at the two examinations (12 respectively 13 mmol/l) The relation of V_{O_2} max to HV is shown in Fig 4

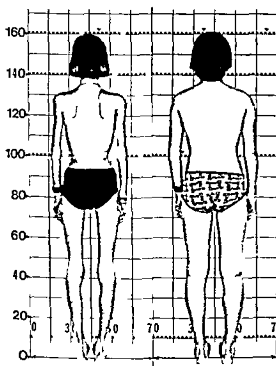


Fig 1 A girl with AN and four months later after weight gain of 17 7 kg

DISCUSSION

The effects of malnutrition on body composition and structure are well known from experimental studies (16) and involuntary incidents in human life (4) The self induced starvation in AN however differs in many important respects from other forms of undernutrition AN is characterized by voluntary caloric restriction which is often combined with an increase in caloric expenditure through physical activity There are seldom any signs of electrolyte disturbances protein and vitamin deficiency and anemia (3 7)

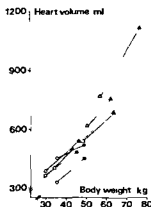


Fig 2 Heart volume in relation to body weight in eight patients with AN O=Girls Δ=boys before rehabilitation Filled symbols after rehabilitation = 109 healthy children (21)

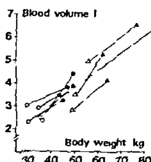


Fig. 3. Blood volume in relation to body weight in eight patients with AN. ---=75 ml/kg b.wt. (15). Other symbols as in Fig. 2.

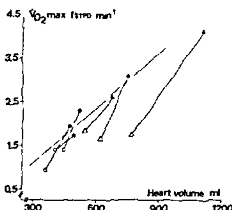


Fig. 4. Maximal oxygen uptake in relation to heart volume. ---=Data from healthy children (21). Other symbols as in Fig. 2.

It is obvious that caloric restriction in the pubertal period can seriously affect growth. None of the pubertal girls showed any increase in height but the observation period after weight gain was too short for any conclusions to be drawn about catch up of growth. However, we have seen other cases of prepubertal girls with long-standing AN in whom growth ceased. Moreover, no girl menstruated during this study but according to Frisch and McArthur (13) all the girls were at the lower limit of weight-to-height for menstruation. Cessation and resumption of menstruation are well known to be correlated to nutritional status (6).

The change in circulatory dimensions in AN was proportional to the reduction in weight. Thus HV and BV showed the same relationship to body weight before and after rehabilitation. Our findings partly contrary to the results of Keys et al. (16) whose studies of volunteer subjects after 24 weeks of semi-starvation showed a weight loss of approximately 24%. They found that BV was little affected by semi-starvation following weight loss. However it is clear that any change in BV may be influenced by the age of onset, the duration of the disease and the degree of physical activity. The duration of the weight loss in these patients averaged one year (range 0.6–1.5).

The semi-starvation in AN has a considerable effect on body composition. *The decline in body weight was due not only to a reduction in fat but also to a decrease in soft fat-free tissue.* LBM increased significantly in all patients after recovery.

Another striking effect of undernutrition in AN was the reduction in V_{O_2} max. This was decreased *more than expected from the change in body size and composition* as previously reported (8, 11). *The V_{O_2} max returned to normal in all patients.* After recovery, the mean increase in the V_{O_2} max/kg b.wt. was 46% for the boys and 16% for the girls. After rehabilitation one boy (patient 1) trained very hard with long-distance running and his

Table IV. *Maximal exercise data before (I) and after treatment (II)*

Pat. no.	Work load (W) I	Work load (W) II	Heart rate (beats/min) I	Heart rate (beats/min) II	Oxygen uptake (l/min) I	Oxygen uptake (l/min) II	Oxygen uptake (ml/kg×min) I	Oxygen uptake (ml/kg×min) II
1	130	330	175	200	1.75	4.12	31	54
2	140	190	180	195	1.79	2.61	37	41
3	130	230	175	195	1.65	3.10	32	50
4	80	140	170	200	0.94	1.93	34	42
7	110	140	180	185	1.38	1.77	40	41
8	100	130	182	195	1.41	2.31	39	47
Mean	115	193	177	195	1.49	2.64	36	46
±S.D.	23	77	5	6	0.32	0.87	4	5
p	<0.05		<0.01		<0.01		<0.05	

aerobic capacity improved 135% in absolute terms None of the other patients trained systematically during the recovery period

The reduction in (V_{O_2} max) in this study is in good agreement with the results of the Minnesota experiment (16) where a decline in V_{O_2} max/kg b wt of 26% was found in 9 men following a 24% weight reduction Unfortunately the authors did not report the maximal heart rate However the recovery pulse rate in their study was lower than expected after starvation and this could be an indirect confirmation of the low maximal heart rate that has also been found in the AN patients The reasons for the low V_{O_2} max and maximal HR are not clear from the present study It could be argued that poor motivation in this special group of patients makes the maximal aerobic power test unreliable but the high respiratory quotient and blood lactate observed would indicate a work level at or very close to their maximal capacity The reduction of muscle mass which may result in a loss of strength may influence the ability to perform a circulatory maximal work This may be a decisive factor and limit the ability of AN patients to pedal to exhaustion However the low maximal heart rate may also reflect the bradycardia and low habitual temperature at rest which would be in agreement with experimental hypothermia and maximal exercise (9)

It is concluded from this study that the body of the adolescent child with AN has a considerable ability to adapt to varying degrees of caloric supply

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Serum Zinc Concentrations in Finns

F Bjorksten[1] A Aromaa P Knekt and L Malinen[2]

From the Research Institute for Social Security and the Division of Epidemiology and Preventive Medicine Social Insurance Institution Helsinki Finland

ABSTRACT Serum zinc concentrations were measured in 1416 Finns aged 15 years and above and belonging to 18 population groups living in various parts of the country The mean serum zinc concentration was for men 12 7 μmol/l (82 μg/100 ml) and for women 11 7 μmol/l (77 μg/100 ml) Zinc levels varied with sex age, length of fast time of day and geographic area *It is noted that the incidence of* coronary heart disease the occurrence of a number of elements in soil and water, and the zinc level in human sera can in a statistical sense be linked to soil composition in Finland Causal relationships, however, remain obscure

Zinc is a trace element necessary to man (30) Zinc deficiency confirmed by the beneficial effects of oral zinc therapy has been observed to be associated with low serum or plasma zinc levels in i v fed patients (17) and in patients suffering from ulcers or sores (3) malabsorbtion (23) or acrodermatitis enteropathica (25) When sheep or rats are fed diets containing low concentrations of zinc plasma zinc concentrations decline (30) In man a fall in serum or plasma zinc levels also occurs after acute tissue injury regardless of origin (20)

Many investigators have examined the serum or plasma zinc concentration in small healthy population groups (6 11 21 35) In Finland Hernberg et al (12) have studied the serum zinc levels of male industrial workers We have examined a large sample of the general population in order to establish the effects of sex age length of fast and time of day Due to study conditions we also observed the effects of an oral glucose load on the serum zinc concentration

Lack of some trace elements amongst them zinc may be involved in the etiology of hypercholesterolemia and atherosclerosis (37) A low ratio of zinc to cadmium in the kidneys has been associated with hypertension (31) Coronary heart disease (CHD) is very common in Finland (38) and there are large regional variations in its incidence and mortality (26) Therefore we analyzed interpopulation variations in serum zinc levels the association of serum zinc with some CHD risk factors (including hypertension) and the association with CHD itself

PERSONS STUDIED AND METHODS

The persons studied participated in multiphasic screening examinations in 1969–72 We invited all adults aged 15 years or more or a representative sample of adults living within a specified area or in the case of industrial groups persons employed by a certain manufacturer within a specified area We attempted to study unselected samples of the general population Therefore we have not excluded persons with a disease persons whose test data have been outside the normal range persons taking medicines or other groups Average participation was 82% The majority of non participants were persons who had moved away from the area but remained in population registers and persons who were away from home for a long period Random samples of approximately 40 men and 40 women from each of 18 population groups altogether 712 men and 701 women were included in the serum zinc study The 18 population groups lived in 16 different communes in various parts of the country (Table I) The groups were chosen to represent rural urban or semiurban and industrial populations and they were studied at different times of the year (Figs 1 and 3)

Persons participating were instructed to fast for at least 4 hours before arriving at the examination site Participants were then questioned concerning the actual length of fast Since an oral glucose tolerance test was included in the multiple screening study all persons except known diabetics were given an oral glucose load graded according to BSA (60 75 or 90 g glucose) The load was given soon after arrival and blood samples were drawn an hour later Participants remained seated or standing

Casual BP after 5 min rest was measured Serum zinc and cholesterol were determined in all individuals Plasma

Present addresses [1]Hospital for Allergic Diseases Helsinki University Central Hospital SF-00250 Helsinki 25 Finland [2]Hässle Ciba-Geigy Oy SF-00510 Helsinki 51 Finland

Table I *Serum zinc concentration (mean and S D) in 18 Finnish population groups*
Groups are listed by time of study Mean values have been adjusted for age length of fast and time of day

		Serum zinc concentration (μmol/l)					
		Men			Women		
Population group	Commune	*n*	Mean	S D	*n*	Mean	S D
1	Utsjoki (center)	33	13 24	1 86	45	11 73	1 28
2	Utsjoki (Kangasniemi)	47	13 05	1 91	32	12 92	2 15
3	Kittilä	38	13 55	2 05	41	12 90	1 69
4	Kemijarvi	37	13 07	1 97	38	12 12	1 56
5	Ylitornio	39	13 47	2 01	35	12 00	1 57
6	Tornio	40	12 71	1 64	40	11 40	1 77
7	Jaala	38	11 51	1 55	40	11 39	1 73
8	Kuusankoski (urban population)	40	12 30	1 82	40	11 42	1 75
9	Kuusankoski (industrial population)	40	13 16	1 92	39	11 55	1 26
10	Kajaani	39	11 92	1 37	37	11 00	1 27
11	Ristijarvi	40	11 35	1 29	40	11 12	1 26
12	Suomussalmi	39	12 24	1 87	39	11 32	1 97
13	Halsua	39	13 17	1 97	40	11 75	1 82
14	Kannus	40	12 63	1 97	38	12 05	1 28
15	Kokkola	42	13 10	1 76	37	12 07	1 61
16	Joensuu	40	12 52	1 58	40	11 72	1 52
17	Kiihtelysvaara	40	12 13	2 36	40	11 24	1 73
18	Tohmajärvi	40	12 76	1 74	36	11 67	1 20

glucose was determined in those who had received the glucose load Serum triglycerides were assayed in population groups living in the West and East (Fig 1)

The effects of the glucose load were studied in a group of 126 persons different from those mentioned above The group was instructed to fast for at least 12 hours and was then subjected to the glucose load Blood was drawn immediately before and 1 hour after the load Plasma zinc was measured

ay methods

and plasmas were separated frozen immediately and red at −20°C in polyethylene tubes until analyzed lasma glucose (9) serum cholesterol (4) and serum triglycerides (2) were determined within 3 weeks of sampling using various AutoAnalyzer methods

All zinc assays were made in the autumn of 1973 using an automated modification (8) of an atomic absorption method (7) A control serum was analyzed after every eighth sample The results exhibited a coefficient of variation of 4 1% Every 30th unknown serum was reanalyzed on the following working day and these reruns gave a coefficient of variation of 4 2% To study the possibility of water evaporation during storage from the sera sodium concentrations were measured in 5% of the sera Raised sodium concentrations indicating evaporation were not found No measurable zinc could be extracted with 50 mmol/l HCl or with serum from any piece of equipment coming into contact with the samples

Statistical methods

In estimating the effects of the oral glucose load a model was assumed where the measurement scale one hour after the load could differ from that before the load by a multiplicative and an additive constant (14) Fisher s *F* test was used to determine whether the multiplicative constant differed from 1 and the additive constant from 0

Regression analysis was used to compute various adjusted (age time of day length of fast population group) zinc means Each group was represented by an (0 1) explanatory variable in the regression analysis (33) The statistical significance of the differences of the adjusted zinc levels and the interactions between length of fast and time of day were tested by means of Fisher s *F* test

The statistic g_1 was used as a measure of skewness (5) When testing differences between sexes the usual *t* test was used

RESULTS

Effects due to a glucose load fasting and diurnal variations

The plasma zinc concentration was studied in a group of 126 persons immediately before and 1 hour after an oral glucose load The mean before the load was 13 4 μmol/l (87 2 μg/100 ml) the correlation coefficient was 0 855 and the best linear equation describing the dependence of the two results was $y=0\ 955x$ where y is the plasma zinc concentration 1 hour after and x the concentration before the glucose load

Results were adjusted for an observed diurnal variation using estimates from a separate study on 576 persons to whom no glucose load was given and who had fasted at least 12 hours (Bjorkstén et al

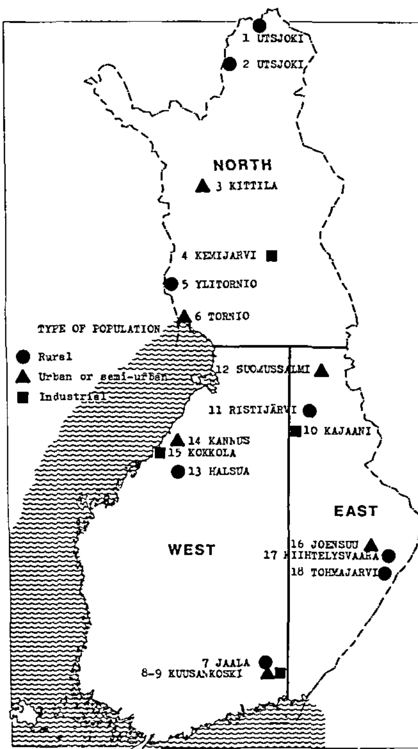


Fig 1 Population groups studied and their classification according to type of population and geographic area

Table II *Effects of fasting on serum zinc concentrations*

Mean values have been adjusted for age time of day and population group (commune) The significance of differences in means was tested with the *F* test

	Length of fast			Zinc (μmol/l)	
	Range (h)	Mean (h min)	No of persons	Mean	*p*
Men	0– 4	4 21	209	12 34	
	5– 8	6 01	132	12 48	<0 01
	9–11	10 44	98	13 08	
	12–	12 59	272	12 84	
Women	0– 4	4 26	227	11 37	
	5– 8	6 02	168	11 72	<0 01
	9–11	10 48	46	11 92	
	12–	13 01	256	12 02	

unpublished results) After this adjustment the glucose load was found to reduce plasma zinc concentrations by 3 9% ($p<0\ 025$)

In the main part of our study 97 3% of the persons were subjected to an oral 1 hour glucose load before the sample for the serum zinc determination was drawn In the following we will assume however that the effects of the glucose load were unimportant

When the length of fast increased from about 4 hours to about 11 hours the increase in the serum zinc mean was 6 0% in men and 4 8% in women (Table II)

een the periods 9–10 a m and 1–3 p m an drop in mean serum zinc concentration was in men when data adjusted for the length f fast were used The values dropped in women by 3 7% but this was not significant (Table III) No interaction was found between the effect of fasting and the diurnal variation

Variations with sex age and type of population

Men had higher mean serum zinc concentration than women in all 10-year age groups below 60 ($p<0\ 05$ for adjusted means in the age group 50–59 $p<0\ 01$ in groups below 50) Both in men and women the values decreased with increasing age (Fig 2 Table IV)

In women both the standard deviation and the skewness of the frequency distribution appeared to decrease with increasing age A positive skewness significantly different from 0 was found in some sex and age groups

Mean serum zinc concentrations in rural urban or semiurban and industrial population groups did not differ significantly (Table V)

Differences between geographic areas and seasonal variation

Serum zinc levels were highest in North Finland and lowest in the East There was also some seasonal variation with low values during the second quarter of the year (Table V) Population groups in West Finland were studied only during the third and fourth quarters of the year and groups in East Finland during the first and second quarters Studies in North Finland were performed more evenly over the year (Fig 3) Therefore the differences in serum zinc concentrations between East and West can be due either to seasonal variation or true geographic variation However geographic variation with highest zinc concentrations in the North remained even after adjustment for time of year Data for individual population groups are given in *Table I*

Serum zinc concentrations and coronary heart disease

To study the association between high BP and low serum zinc levels in our population groups we defined a systolic BP of ≥160 mmHg as high and serum zinc levels of <11 μmol/l in men and <10 5 μmol/l in women as low In view of the similar age structure in our populations the data were not age standardized High systolic pressures occurred more often in population groups with a large proportion of low serum zinc levels The rank correlation coefficients were 0 51 in men (significantly dif

Table III *Diurnal variations in serum zinc concentrations*

Mean values have been adjusted for age length of fast and population group (commune) The significance of differences in means was tested with the *F* test N S =not significant

	Time of day	No of persons	Zinc (μmol/l) Mean	*p*
Men	before 10 a m	113	13 29	
	10–12 a m	424	12 70	<0 01
	1– 3 p m	174	12 13	
Women	before 10 a m	75	12 06	N S
	10–12 a m	366	11 74	0 05<*p*<0 1
	1– 3 p m	256	11 61	

ferent from 0 $p<0.01$) and 0 24 in women No significant association between the use of diuretics and serum zinc levels which might have affected the above conclusions could be found However women aged 50 and above who used diuretics had slightly (0 35 μmol/l) lower serum zinc means than the corresponding non users

There were some low level correlations between the serum zinc concentration and several other CHD risk factors (Table VI) These associations were further explored within age and population groups The highest correlation coefficients were observed between serum zinc and triglycerides in men aged 15–49 in population groups 13–15 in West Finland ($r=-0.20$) and 16–18 in East Finland ($r=-0.22$) Both coefficients were significantly different from zero ($p<0.05$)

Use of digitalis and a history of CHD were not associated with serum zinc levels Neither was mortality from any cause nor from CHD associated with low zinc concentrations as indicated by case control analysis with a 4-year follow up period However there were only 16 persons who had died of CHD

DISCUSSION

Serum zinc levels and their dependence on sex and age

We found a mean serum zinc concentration of 12 7 μmol/l (82 μg/100 ml) in Finnish men and 11 7 μmol/l (77 μg/100 ml) in Finnish women These levels are lower than many other reported values which however are quite variable (6 11 21 35) In Finland Hernberg et al (12) have previously observed plasma zinc means of about 18 μmol/l (119 μg/100 ml) in men In our study the inclusion of elderly persons and of persons studied in the after noon and also the effects of the glucose load tend to lower the zinc levels This together with the contamination risk and other factors which affect laboratory accuracy (bias) make it hazardous to compare mean values reported in different studies (35)

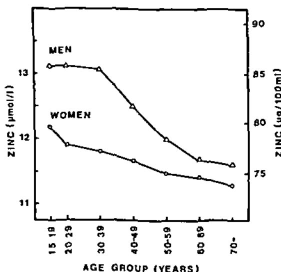


Fig 2 Variations with sex and age in mean serum zinc concentrations adjusted for length of fast

Table IV *Variations with sex and age in serum zinc concentrations*

Mean values original and adjusted for length of fast are given The significance of differences in adjusted means was tested with the F test

	Age (y)	No of persons	Mean zinc concentration (μmol/l) Original	Adjusted	p	S D
Men	15–19	69	13 22	13 12		1 72
	20–29	157	13 13	13 12		1 89
	30–39	171	13 05	13 07		2 07
	40–49	133	12 57	12 49	<0 01	2 02
	50–59	86	11 90	12 00		1 85
	60–69	62	11 57	11 68		1 78
	70–	34	11 68	11 60		1 54
Women	15–19	79	12 24	12 18		2 02
	20–29	161	11 91	11 90		1 79
	30–39	131	11 82	11 80		1 64
	40–49	134	11 61	11 66	<0 025	1 67
	50–59	87	11 47	11 47		1 39
	60–69	74	11 40	11 41		1 54
	70–	38	11 42	11 28		1 33

We found that men under 60 had higher serum zinc concentrations than women In both men and women the concentration fell with increasing age (Fig 2 Table IV) Analogously Lindeman et al (21) and Heinemann (11) have found higher plasma zinc levels in men than in women and Lindeman et al also described a decrease with increasing age

Diurnal variations fasting and the effect of a glucose load

Morning serum zinc concentrations tended to be higher than afternoon values This diurnal variation was however significant only in men (Table III) In previous small studies similar (3 7 13) or different (19) diurnal variations have been observed

Table V *Variations in serum zinc concentrations with time of year type of population and geographic area*

Mean values original and adjusted for age time of day and length of fast are given The significance of differences in adjusted means was tested with the *F* test N S =not significant

		Men				Women			
			Mean zinc concentration (µmol/l)				Mean zinc concentration (µmol/l)		
Criteria for grouping populations	No of popula tions	*N*	Origi nal	Ad justed	*p*	*N*	Origi nal	Ad justed	*p*
Time of year (quarter)									
1st	4	155	12 51	12 85		150	11 70	11 71	
2nd	5	199	12 15	12 23		195	11 31	11 36	
3rd	4	150	12 65	12 61	<0 01	163	11 70	11 75	<0 01
4th	5	207	13 26	12 97		189	12 22	12 11	
Type of population									
Rural	8	316	12 44	12 60	N S	308	11 78	11 71	N S
Urban or semiurban	6	237	12 81	12 64	>0 1	238	11 71	11 80	>0 1
Industrial	4	158	12 87	12 80		151	11 66	11 66	
Geographic area									
North	6	234	13 36	13 18		231	12 30	12 11	
West	6	239	12 73	12 65	<0 01	234	11 60	11 72	<0 01
East	6	238	11 90	12 16		232	11 29	11 36	

We found that the mean serum zinc concentration in both men and women increased with the length of fast (Table II) This may be explained by a relative dehydration leading to a lowering of plasma vol ume Some earlier authors found that meals de creased serum or plasma zinc concentrations (3 16) while others found no such effect (24) These ous studies were made in small groups of 3–12 ons which may explain the conflicting results

Plasma zinc concentrations measured 1 hour after an oral glucose load were 4 5% lower than those before the load Part of this reduction was due to the diurnal variation but a significant 3 9% reduc tion could be attributed to the glucose load Similar observations have been reported previously (6 27) The change may be due in part to changes in plasma volumes in part to metabolic alterations

Table VI *Partial (constant age) correlation coeffi cients for the serum zinc concentration on coronary heart disease risk factors*

	Men		Women	
Risk factor	*N*	*r*	*N*	*r*
Systolic BP	712	0 004	704	−0 009
Diastolic BP	712	0 042	704	0 063*
Plasma glucose 1 h after load	689	−0 139**	689	−0 149**
Serum cholesterol	712	0 096 *	704	0 085*
Serum triglycerides	477	−0 112**	468	−0 020

* Significantly different from 0 ($p<0\ 05$)
** Significantly different from 0 ($p<0\ 01$)

Geographic area and coronary heart disease

The incidence of CHD among men in East and Central Finland is the highest recorded anywhere In Finland the lowest incidence is found in coastal regions and in the South–West (29) Soils, including cultivated soils in southern and western coastal regions are largely fine grain mineral soils e g clay and silt In eastern and central Finland coarse min eral soils, e g sand and moraine dominate In northern Finland cultivated soils are mainly organic (peat) or coarse mineral soils (18) In the waters of East and Central Finland many elements are found in lower concentration than elsewhere presumably due to poor dissolution from coarse mineral soils (10 22 28 32 36) The same is true of some ex tractable soil elements e g magnesium (15 18) There is an obvious statistical association between regional disease rates soil composition and water composition

In the present study we have examined zinc con centrations in serum rather than levels in water and

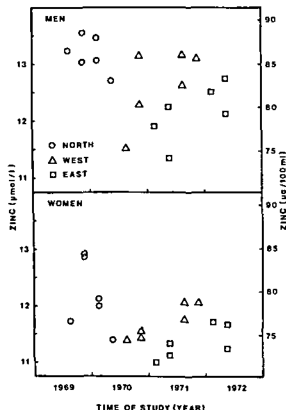


Fig 3 Mean serum zinc concentrations as a function of sex geographic area and time of study

soil but the results are reminiscent of those referred to above The lowest serum zinc levels were found in East Finland (Table V) where the CHD mortality figures are highest Also factors other than soil composition can be suggested as ultimate causes of the observed distribution of serum zinc levels We studied population groups in the East and the West at different times of the year and the observed East–West difference could be due to a hypothetical seasonal variation (Fig 3) In an extensive study Seppanen and Takkunen (34) have shown that the diet in North Finland where serum zinc levels were highest contains more meat than diets elsewhere in the country As a consequence absorbable iron intakes were highest in the North The same could be true for absorbable zinc which would explain the relatively high serum zinc levels in North Finland

In previous Finnish studies the prevalence and incidence of CHD have been correlated with the mineral content of the drinking water of individual subjects In such studies a negative correlation has been found with chromium (28) fluorine (22) and magnesium (22) and a positive correlation with copper (28) However no causal inferences can be drawn on the basis of these results

We found that hypertension was more common in population groups with a high proportion of persons with low zinc values On the level of individuals there was little evidence for such an association The protective effect of zinc against cadmium induced hypertension could be poorer in the East where hypertension is more prevalent than elsewhere (1)

We observed a modest negative correlation between the serum zinc and serum triglyceride concentrations of individual subjects No association was found between low serum zinc and total mortality CHD mortality use of digitalis or a history of CHD

In conclusion the most interesting of our observations may be the presence of regional differences in serum zinc concentrations in Finland We also note that serum zinc levels CHD and hypertension figures and the levels of a number of elements in soil and water can in a statistical sense be linked to soil composition in Finland However causal relationships between these variables remain obscure

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Taste Dysfunction and Changes in Zinc and Copper Metabolism during Penicillamine Therapy for Generalized Scleroderma

Lone Knudsen and Kaare Weismann

From the Department of Dermatology University of Copenhagen Rigshospitalet Copenhagen Denmark

ABSTRACT The taste function and the zinc and copper levels in serum and urine were followed for up to 16 weeks in ten patients who were started on penicillamine therapy for generalized scleroderma (9 patients) and rheumatoid arthritis (one patient) During therapy the serum zinc concentration remained unchanged, whereas the serum copper concentration increased significantly during the first 4–5 weeks and then tended to decrease Urinary copper rose significantly and remained considerably above the upper normal limit throughout the study Six of the patients complained after about 4–5 weeks of a decreased taste function which was gradually restored whether the medication was stopped or continued The alterations in the taste acuity for sweet salt, sour, and bitter significantly paralleled the variations in urinary copper before as well as during therapy Thus the patients who showed the most pronounced loss of taste had a lower urinary copper output than those whose taste acuity was less disturbed

In 1943 Abraham et al (1) isolated penicillamine (β β dimethylcysteine) as a hydrolysate of penicillin Its chelating effects have been utilized for hepatolenticular degeneration (Wilson s disease) (30) for lead poisoning (3) and cystinuria (6 7) About a decade ago penicillamine was introduced for generalized scleroderma (2 4 11 14 28) and rheumatoid arthritis (10 15 17 21 25) In scleroderma the mode of action was ascribed to an inhibitory effect on the synthesis of collagen (2 4 11 14 28) In rheumatoid arthritis the effect was ascribed to immunosuppression (10 17) inhibition of an antigen stimulation (17) or to an antiinflammatory action in the connective tissue (10)

Serious side effects of penicillamine include leucopenia aplastic anaemia nephritis drug fever and cutaneous eruptions (8 16 17 22 25 31) Loss of taste is a discomforting and untoward effect of penicillamine which occurs in 25–35% of the patients except those with Wilson s disease in whom it develops in only 4% of the cases (12 13 16 18) The cause of the taste dysfunction during penicillamine treatment has been attributed to copper or zinc depletion (7 12 13 16) whereas taste dysfunction associated with other disease states has been ascribed to zinc deficiency (26) However oral supplementation with the elements has given inconsistent results (12 13 21 25 26) The taste dysfunction during penicillamine therapy usually improves spontaneously even if the treatment is continued and always normalizes when the medication is stopped (12 13 21)

To clarify the role of zinc and copper in the penicillamine induced taste dysfunction we followed the early changes in the taste function and the alterations in the metabolism of zinc and copper in patients receiving penicillamine for generalized scleroderma and rheumatoid arthritis

PATIENTS AND METHODS

Eighteen patients were initially included in the study but as eight of them were unable to show up for regular control visits they were omitted The remaining ten patients five men and five women 35–74 years old were studied They were all started on penicillamine chloride (Dimetyl cystein® capsules Dista) 450–900 mg daily for generalized scleroderma (9 patients) and rheumatoid arthritis (1 patient) The disease state had lasted for 3 months–19 years Half of the patients had not received any thera

Address for reprint requests L. Knudsen MD Department of Dermatology Rigshospitalet DK 2100 Copenhagen Denmark

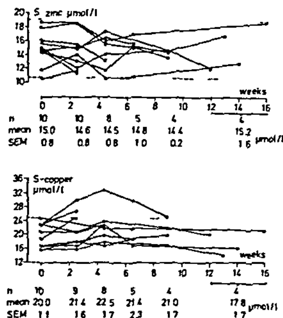


Fig 1 Variation in S-Zn and S-Cu concentrations during penicillamine treatment (n=no of patients + denotes that the level is statistically significantly higher than the previous level ($p<0.05$) —— =reference ranges)

py whereas the other half had received either gold preparations glucocorticoids azathioprine penicillamine or ibuprofen Apart from the patient with rheumatoid arthritis who was receiving 15 mg of prednisone daily none of the patients had been given any treatment for the last year

Blood and urine samples were taken before and during the study at intervals of 2-3 weeks for a total of four months Serum zinc (S-Zn) serum copper (S-Cu) urinary zinc excretion/24 h (U Zn) and urinary copper excretion/24 h (U-Cu) were determined by atomic absorption spectrophotometry (Medicinsk Laboratorium Copenhagen) The reference ranges are S-Zn 10 6–18 9 µmol/l S-Cu 12–25 µmol/l U Zn 1 5–10 7 µmol/24 h (according to Underwood (29)) U Cu 0 1 1 3 µmol/24 h Furthermore total S-protein P albumin S-creatinine Hb concentration WBC and thrombocyte count ESR and urinalysis for protein glucose and Hb were performed Endogenous creatinine clearance/24 h was determined before the therapy was started

The taste function for sweet salt sour and bitter was evaluated semiquantitatively according to Krarup (20) as modified by Zilstorff (33) Three scores were obtained if the patient could detect the lowest concentration of sucrose (4% aqueous solution) sodium chloride (2 5%) citric acid (1%) and quinine chloride (0 075%) Two scores were given if the tastant was identified by the higher concentrations (10 7 5 5 0 and 0 5% respectively) and one score if only the highest concentrations were recognized (40 15 10 and 1% respectively) If the patient was unable to identify the tastant no score was obtained

For the statistical analyses performed by Aa. Vølund Wilcoxon s rank sum test for paired data Spearman s rank correlation coefficient and the product moment correlation coefficient were applied

RESULTS

The S Zn and S Cu levels during penicillamine therapy are shown in Fig 1 S Zn remained practically unchanged whereas S-Cu was increased during the first 9 weeks with a maximum after 4–5 weeks During the fourth month of the trial S-Cu showed a decreasing tendency but this did not reach statistical significance at the 5% level probably as a consequence of the small number of patients at the end of the study In one patient S Cu was remarkably high during the first 6–7 weeks of therapy

The U Zn and U Cu excretions are presented in Fig 2 The pretreatment U Zn values were higher than normal in seven of the ten patients studied The significance of this finding remains unknown U Zn tended to increase during the trial but the changes were not statistically significant U-Cu was significantly increased after 2 3 weeks and after 4–5 weeks and remained elevated throughout the time of observation Individual differences in U Cu were notable especially after 2–3 weeks and to a lesser degree after 4–5 weeks One patient excreted about ten times the normal amount of U Cu after 2–3 weeks and 9 weeks

The S-Zn concentrations were significantly correlated to the P albumin concentrations Before

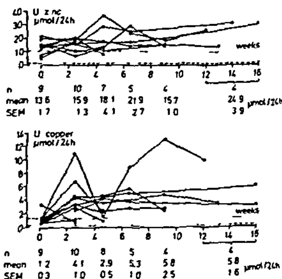


Fig 2 Variation in U Zn and U-Cu excretion during penicillamine treatment Symbols as in Fig 1

Table I *Mean scores for four tastants during penicillamine therapy*

Week	n	Sweet	Salt	Sour	Bitter
0	10	2 1	2 3	2 0	2 2
2–3	10	1 8	2 0	2 1	2 5
4–5	8	1 9	1 9	2 0	2 3
6–7	5	1 8	2 2	2 2	1 8
8–10	4	2 0	2 5	2 0	2 3
11–16	4	2 5	2 5	2 3	2 3

therapy the product moment correlation coefficient r_p was 0 79 ($p<0\ 05$ $n=7$) The relationship follows the equation $y=9\ 9+0\ 010\ x$ in which y is the S Zn concentration (μmol/l) and x the P albumin concentration (μmol/l)

After about one month s treatment six of the ten patients spontaneously complained of a decreased taste function which was confirmed by the taste function tests (Table I) Thus mean scores for the tastants were decreased after 2–7 weeks but practically normal after 8–10 weeks therapy The normalization paralleled a subjective improvement of the taste function both in the patients who continued to receive penicillamine ($n=3$) and in those who stopped taking the drug ($n=3$) The changes in the taste function test did not reach statistical significance probably as a consequence of the limited number of patients included in the study

The correlations between the changes in the taste function and the S-Zn S-Cu U Zn and U-Cu levels were analysed by Spearman s rank correlation coefficient this being preferred because the quantities might not follow a Gaussian distribution Only correlations involving the U-Cu excretions were found to be statistically significant at the 5% level (Fig 3) Thus after 2–3 weeks and 4–5 weeks U-Cu correlated positively with the changes in scores for salt from 0 to 2–3 weeks and to the changes in scores for sweet from 0 to 4–5 weeks respectively The pretreatment U-Cu levels correlated with the changes in scores for sour from 0 to 2–3 weeks and from 0 to 4–5 weeks and with the changes in scores for bitter from 0 to 4–5 weeks This means that the taste function was better in the patients with a high U-Cu output than in those with a low U-Cu excretion who had the most pronounced loss of taste It should be noted that a large number of correlations were calculated and that the significant [illegible] in Fig. 3*b* was due to a low U-Cu excretion [illegible] the two patients who showed a marked [illegible] the taste function The correlations involving pretreatment U Cu (Fig 3*c* *d* and *e*) were due to relatively small differences in the U Cu output During therapy when U Cu increased the close correlation between U Cu and taste function seems to have disappeared The most plausible of the significant correlations is shown in Fig 3*a*

In three patients the treatment was stopped after four weeks because of influenza like symptoms accompanied by an exanthema in one patient and by diarrhoea in another Another three patients had similar symptoms which gradually disappeared after the penicillamine dose had been reduced from 900 to 450 mg daily Only two of the ten patients studied had no side effects at all After about 5 months therapy penicillamine was withdrawn from two patients because of proteinuria and thrombopenia respectively In the rest of the patients the laboratory analyses remained normal

DISCUSSION

The present results suggest that alterations in copper metabolism are involved in the taste dysfunc

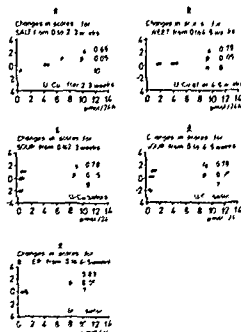


Fig 3 [illegible] correlations between [illegible] scores [illegible] the tastants and the U-Cu excretion [illegible] therapy [illegible]

tion which occurs during penicillamine therapy whereas the role of zinc seems uncertain. The patients who had the lowest U-Cu excretions before and during the therapy showed the most pronounced loss of taste when compared with the patients who excreted larger amounts of copper in the urine. The interpretation of the observed significant correlations, which include all four tastants, remains speculative, since a causal relationship can only be suggested and not proven by statistical means. The following two hypotheses are suggested. According to Henkin et al. (13), the taste dysfunction during penicillamine therapy is attributable to copper depletion due to an increased urinary copper output. According to this theory, the low U-Cu output in the patients who had a severe loss of taste is due to these patients having less copper available for complexing with penicillamine. Thus, even a small increase in the urinary copper excretion mediated by penicillamine would lead to a copper deficiency in the tissues, including the taste organs. This explanation, however, does not fit very well with the fact that in this study S-Cu increased during the first weeks of penicillamine therapy. Neither does the theory explain the transitory nature of the taste dysfunction, which one would expect to continue or be aggravated during prolonged penicillamine therapy. The latter is believed to cause copper depletion, as reflected by a decreased S-Cu level and an increased U-Cu output (10, 24). An alternative explanation seems more plausible to us. After initiation of penicillamine medication, the S-Cu level increases due to enhancement of the intestinal copper uptake (24). This might lead to an accumulation of copper, which is not eliminated by the kidneys until some weeks later, as shown in this study (Fig. 2). The peak of taste dysfunction occurred after about 4–5 weeks, when S-Cu was at about the highest level but before U-Cu had reached its maximum. Later on, as S-Cu decreased and U-Cu was still increased, the taste function improved spontaneously. This might indicate that a copper excess rather than a copper deficit is involved in the penicillamine-induced taste dysfunction. In accordance with this, patients with a decreased capacity to eliminate the copper excess would thus show the most pronounced loss of taste.

Hansen et al. (10) noted a raised S-Cu before penicillamine therapy for rheumatoid arthritis, which after 6 months was found to be subnormal. An identical course was observed in our study, but as a consequence of the small number of patients present at the end of the trial, the changes were statistically insignificant. The findings are in agreement with the observation that long-term penicillamine therapy might lead to copper depletion (13). After several months of therapy, however, the taste dysfunction is generally restored spontaneously.

Loss of taste was reported to occur in hypozincaemic patients with coeliac sprue (27) and thermal burns (5), in children with anorexia, poor growth and low hair zinc (9), and in patients receiving penicillamine for cystinuria (7). Apparently, it is never a complaint of patients suffering from acrodermatitis enteropathica or the zinc depletion syndrome due to long-term parenteral feeding, in whom severe zinc deficiency may be present (32). We found no significant changes in the S-Zn levels or the U-Zn excretions during penicillamine therapy, although the U-Zn excretions in the patients who were studied after 12–16 weeks' therapy were higher than the initial values. This probably reflects an enhanced zinc uptake from the gut mediated by penicillamine (24), and not a negative zinc balance causing mobilization of tissue zinc, which might lead to zinc depletion. This, however, may occur under special circumstances, as reported during prolonged high-dose penicillamine therapy in a patient with Wilson's disease who developed zinc deficiency with characteristic skin manifestations, including total alopecia (19).

Most clinical studies dealing with loss of taste during penicillamine therapy are based on various patient groups, and the duration of therapy as well as the penicillamine dosage are often not accounted for. Therefore, it is difficult to compare the results with those of the present investigation, which, to our knowledge, is the first to delineate the changes in zinc and copper metabolism and taste function during the initial phase of penicillamine therapy. More extensive and prolonged controlled clinical studies including supplementation with zinc and copper should be performed to clarify the role of these elements in the taste function.

ACKNOWLEDGEMENT

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Vitamin B_{12} Body Stores during Oral and Parenteral Treatment of Pernicious Anaemia

Ragnar Berlin Hans Berlin Gunnar Brante and Åke Pilbrant

From the Department of Internal Medicine Linkoping University Medical School Linkoping the Department of Clinical Chemistry St Lars Hospital Lund and AB Kabi Stockholm Sweden

ABSTRACT Oral treatment of pernicious anaemia patients with 1 mg cyanocobalamin daily has been shown before to be as effective as conventional injection therapy The result of this study indicates that oral treatment also keeps the vitamin B_{12} body stores adequately filled, a confirmation of earlier results obtained in another way

Since the introduction in Sweden in 1964 of the oral treatment of pernicious anaemia with large daily doses (1 mg) of vitamin B_{12} without intrinsic factor (Behepan® tablets) this regimen has been widely accepted as an equivalent alternative to conventional intermittent injection therapy It has also proved effective in maintaining long term remissions as demonstrated by clinical and laboratory criteria (4)

The very slow decrease in the serum B_{12} concentration after the withdrawal of oral medication in a number of patients indicates that this treatment also keeps the depots adequately filled (4) However the degree of replenishment of the B_{12} body stores during oral and parenteral treatment has not been subjected to direct comparisons To get an idea of the degree of filling of these depots Bastrup Madsen (1 2) followed up the disappearance rate of vitamin B_{12} in serum after a single injection of a sustained release preparation Betolvex® which is a cyanocobalamin–tannin complex suspended in a sesame oil–aluminium monostearate gel containing 1 mg B_{12} per ml After performing this experiment in normal subjects in pernicious anaemia patients in relapse and in pernicious anaemia patients maintained on hydroxocobalamin therapy or on Betolvex for two years he concluded that a low serum B_{12} disappearance curve indicates more or less empty B_{12} stores while a curve similar to that in normal subjects would mean adequately filled depots He emphasized that this method can give only a rough estimation of the degree of replenishment or depletion of the vitamin B_{12} body stores but that it can still be used to compare different vitamin B_{12} preparations in this respect

In order to compare vitamin B_{12} depots after long term treatment of pernicious anaemia patients with cyanocobalamin tablets and with hydroxocobalamin injections we decided to adopt the Bastrup-Madsen technique and made the following study

PATIENTS AND METHODS

Eleven pernicious anaemia patients treated for many years orally (1 mg cyanocobalamin daily mean 8 4 years) and 10 patients treated with hydroxocobalamin injections (1 mg every third month mean 8 0 years) were included in the study The diagnosis was confirmed by conventional laboratory procedures including bone marrow examinations and serum B_{12} determinations as well as by a satisfactory therapeutic response Seven healthy subjects *matched with the orally and parenterally treated patients* with respect to age and sex were used as controls

To ensure as uniform conditions as possible the orally treated patients were instructed to discontinue therapy for a period of two weeks before the study The parenterally treated group was invited to enter the trial immediately before their ordinary check up—as a rule about three months after the preceding injection The healthy subjects entered the study without any extra precautions with regard to dietary habits etc

In order to get an idea of the reliability of the experimental design we also studied nine untreated pernicious anaemia patients In these cases the vitamin B_{12} body stores must be considered empty since with one exception the patients were all severely anaemic and had very low serum B_{12} levels In two cases slight neurological symptoms of vitamin B_{12} deficiency were also noted

Blood was drawn from an antecubital vein into Vacutainer tubes without an anticoagulant for serum B_{12} de

terminations Immediately afterwards 1 ml of Betolvex was injected i m Blood samples for vitamin B_{12} determinations were drawn after 24 hours and thereafter at weekly intervals for four weeks and every two weeks for an additional four week period No other treatment was allowed during the entire test period

For obvious reasons the most severely anaemic patients were followed up for only 3–4 weeks after the single injection of Betolvex after which time they were subjected to regular therapy The vitamin B_{12} determinations were performed at the Laboratory of Clinical Chemistry of the Linkoping University Medical School in accordance with an isotope dilution method developed by Tibbling (5) and they were carried out by the same experienced technician throughout the study Normal range for this method is 150–900 ng/l (110–660 pmol/l)

The statistical calculations were done according to generally accepted methods

RESULTS

The disappearance curve for the healthy subjects is shown in Fig 1 together with the corresponding curves for the patients treated with hydroxocobalamin injections and with cyanocobalamin tablets All three curves show an obvious conformity and as indicated in Table I the differences between the three groups are not statistically significant with regard to vitamin B_{12} values at different times B_{12} elimination rates and areas under the curves

In contrast the pernicious anaemia patients in clinical and haematological relapse (bottom curve in Fig 1) did not show as high levels of serum B_{12} as healthy subjects and the adequately treated patients After 24 hours the values had already dropped to significantly lower levels than for the other three groups and then remained lower during the observation period also the area under the curve was significantly smaller

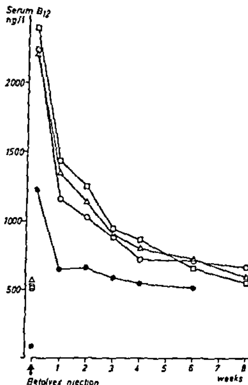


Fig 1 Serum B_{12} levels after injection of 1 mg cyanocobalamin in sustained release form (Betolvex®) □—□=Pernicious anaemia patients treated for several years with 1 mg hydroxocobalamin i m every third month Last injection about 3 months before the study (n=10) △—△=Pernicious anaemia patients treated for several years with cyanocobalamin tablets (Behepan®) 1 mg daily Medication discontinued two weeks before the study (n=11) ○—○=Healthy subjects (n=7) ●—●=Untreated pernicious anaemia patients (n=9)

DISCUSSION

The first conclusion to be drawn from our study is that the method of Bastrup Madsen seems to be suitable for disclosing major differences in the degree of filling of the vitamin B_{12} stores in the body The serum level curve after a Betolvex injection is significantly lower for the untreated patients than for the adequately treated ones and for healthy subjects The relative level of these curves might be interpreted as an indirect manifestation of the remaining binding capacity of the vitamin B_{12} stores

It appears from our study that the orally treated patients had got their vitamin B_{12} body stores filled to the same extent as healthy subjects This holds true also for the patients treated with three monthly injections of hydroxocobalamin In addition we have three patients treated every third month with Betolvex (Table II) and their mean vitamin B_{12} curve after a Betolvex injection is similar the area under the curve does not seem to differ from those of the other three groups and the differences in serum B_{12} concentration are small if any

To some extent our results are at variance with those reported by Bastrup-Madsen (1 2) the serum B_{12} levels in his group of healthy subjects and especially in his Betolvex group are considerably higher than in our corresponding groups The curves for untreated pernicious anaemia patients are very similar in both studies On the other hand

Table I *Serum B_1 (ng/l) after injection of Betolvex® (cf Fig 1)*

	Time after injection							
	0	1 day	1 week	2 weeks	3 weeks	4 weeks	6 weeks	8 weeks
1 *Pernicious anaemia patients treated with hydroxocobalamin i m 1 mg/3 months*								
Mean serum B_{12}	520	2 400	1 440	1 250	940	870	660	550
S D	130	1 040	600	540	330	250	150	110
N	10	9	10	10	10	10	10	10
2 *Pernicious anaemia patients treated with cyanocobalamin orally 1 mg/day*								
Mean serum B_{12}	560	2 200	1 350	1 140	910	800	730	600
S D	190	690	470	310	220	240	180	180
N	11	11	11	11	11	11	11	11
3 *Healthy subjects*								
Mean serum B_{12}	510	2 240	1 160	1 030	880	720	710	670
S D	180	1 270	310	170	200	70	120	170
N	7	7	7	7	7	7	6	7
4 *Pernicious anaemia patients untreated*								
Mean serum B_{12}	80	1 230	650	660	590	550	510	–
S D	30	600	260	310	260	260	250	–
N	9	8	9	8	8	8	5	–

Statistical analysis

Mean serum B_{12} levels Between groups 1 2 and 3 there are no significant differences between any pairs at any time Differences between group 4 and groups 1–3 are significant for all pairs up to 3 weeks also the 4-week values are different (the difference between group 4 and group 3 at 4 weeks is just below the 95% level of significance however) At 6 weeks the difference between group 4 and the others is significant only for one pair out of three (Note the small number of patients remaining in group 4 after 6 weeks!)

Mean areas under curves Between groups 1 2 and 3 there are no significant differences when areas for 0–4 0–6 or 0–8 weeks are compared Group 4 shows a significantly lower area than all the other three for both 0–4 weeks and 0–6 weeks

Mean elimination rates No significant differences between groups 1 2 and 3 A reliable rate constant for group 4 could not be established

Bastrup Madsen s values for patients on hydroxocobalamin therapy are on an average lower than ours at least up to four weeks

A possible explanation of the latter difference is that Bastrup Madsen had intentionally omitted the customary initial loading doses to his hydroxocobalamin patients and had treated them for only two years when the test was performed (ref 2 and personal communication 1976) This may have been insufficient to achieve full remission including adequate filling of the vitamin B_{12} stores Our patients on the other hand had been treated for an average of eight years

The other differences may be due to other causes the most probable one being different assay methods The Lactobacillus Leichmannii method used by Bastrup Madsen is undoubtedly rather non specific compared with the isotope dilution technique used in our study It is not exactly known which compounds that act on the growth

Table II *Serum B_{12} values (ng/l) after Betolvex injection in three pernicious anaemia patients on long term treatment with Betolvex*

	Time after injection							
	0	1 day	1 week	2 weeks	3 weeks	4 weeks	6 weeks	8 weeks
	380	1 480	1 110	950	690	680	520	380
	510	2 190	1 150	810	820	740	630	400
	410	2 520	940	730	680	610	390	330
Mean	430	2 060	1 070	830	730	680	510	370

of L Leichmannii—vitamin B_{12} metabolites and others—may appear in serum from i m depots of the vitamin B_{12}-tannin complex present in Betolvex Bastrup-Madsen (2) and Bastrup-Madsen et al (3) have also commented on this possible complication

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Glucagon Effects on Plasma Cyclic AMP and other Reactants in Normals and Low Insulin Responders

Bo Israelsson Goran Fex Jorgen Malmquist and Gunnela Norden

From the Departments of Medicine and Clinical Chemistry University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT The metabolic response to i v glucagon was evaluated in 11 normal individuals and 8 healthy low insulin responders Elevations of plasma cyclic AMP and blood glucose were similar in both groups Accordingly, no indications were seen of differing hepatic responsiveness to glucagon In contrast, the groups differed in the course of plasma glycerol during the test

The liver occupies a central position in glucose homeostasis (5) In many cases of diabetes the impairment of glucose tolerance is caused more by hepatic overproduction of glucose than by diminished tissue utilization (5) Inappropriately high hepatic glucose output is related to disturbances in insulin and glucagon levels (5 15) and at least in some cases to a reduction in hepatic insulin sensitivity (6) Conversely individuals with normal glucose tolerance despite a reduction of glucose induced insulin secretion (low insulin responders) may have an increased hepatic insulin sensitivity (2) It is unknown whether some healthy low insulin responders have a decreased responsiveness to glucagon To date disturbed glucagon sensitivity has only been found in uremia where sensitivity is enhanced (16) and in children with low growth hormone production whose glucagon sensitivity is lowered (7)

Glucagon is believed to exert its effect on the liver by increasing the formation of adenosine 3 5 monophosphate (cyclic AMP cAMP) in the hepatocyte (4) The increase in intracellular cAMP is accompanied by an efflux of the molecule Measurements of plasma cAMP can therefore serve to indicate changes in intracellular levels (1) Most or all of the plasma cAMP increment caused by glucagon derives from the liver (12 18)

The present study aimed to elucidate whether healthy low insulin responders differ from completely normal individuals in their metabolic reactions to exogenous glucagon Plasma cAMP was used to evaluate hepatic responsiveness Plasma glycerol was measured as an index of adipose tissue lipolysis and glycerol elimination Furthermore glucagon induced insulin secretion was monitored and compared with glucose induced secretion

SUBJECTS AND PROCEDURES

Eight low insulin responders and 11 normal individuals were studied All 19 had a normal oral or i v glucose tolerance Criteria for normality were blood glucose <6 5 mmol/l at 120 min in the oral glucose tolerance test (30 g glucose/m² BSA) or a k value >0 9 in the i v test (25 g/m²) All individuals were healthy normal weight (actual/ideal weight 0 92–1 04) males aged 48–50 The insulin response was expressed as the sum of plasma insulin levels obtained during the test procedures A low insulin response was defined as figures below the 5th percentile of response data obtained in a health control survey of the middle aged male population from which the study subjects were drawn

In the morning after 10–12 hours fasting the subjects were given 0 5 mg glucagon (Novo Industries Copenhagen Denmark) as an i v bolus during 30 sec Venous blood was taken into heparin tubes (for glycerol) and EDTA tubes before glucagon 5 min after and at 10 min intervals thereafter

ASSAY METHODS

Blood glucose was determined with glucose oxidase according to Marks (13) Plasma cAMP was measured by the radioligand technique of Tovey et al (19) using the assay kit of the Radiochemical Centre Amersham Bucks

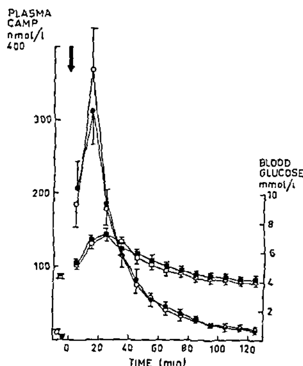


Fig 1 Effects of glucagon 0 5 mg i v (arrow) on plasma cyclic AMP (● ○) and blood glucose (■ □) in 8 low insulin responders (filled symbols) and 11 normals (open symbols) Mean ± S E

UK Plasma glycerol was measured enzymatically by the Laurell and Tibbling procedure (10) and plasma insulin by radioimmunoassay according to Heding (9)

RESULTS

As expected, glucagon injection caused a rapid marked plasma cAMP increase, which was maximal (300–400 nmol/l) at 15 min post injection and returned to the initial level (10–20 nmol/l) at 125 min (Fig 1) Blood glucose rose to a maximum at 25 min and declined to baseline at 125 min (Fig 1) The plasma cAMP and blood glucose increments in the low insulin responders did not differ from those in the normal individuals

Likewise there was no statistically significant difference between groups with regard to insulin response to glucagon (Fig 2)

In all subjects plasma glycerol decreased during the test However at several sampling points glycerol levels were higher in low insulin responders than in normals (Fig 2)

In all 14 subjects who had had an i v glucose tolerance test, the insulin response to glucose was compared with that to glucagon The total insulin areas during 5–60 min post injection were compared There was no correlation between glucose induced and glucagon induced insulin response ($r=0.16$ $p>0.1$)

DISCUSSION

It is believed by many that the primary effect of insulin is to reduce the cAMP elevating effect of glucagon and other hormones (3) In the present context however the glucagon levels obtained were so high that differences between individuals in plasma insulin levels in all probability did not influence hepatic cAMP Accordingly the test is seen as an evaluation of hepatic cAMP production capacity at a strong glucagon stimulus As there was no difference between groups in the responses of cAMP or glucose no difference was found in hepatic glucagon reactivity

Plasma glycerol levels reflect adipose tissue lipolysis rates (8) Lipolysis in the rat adipocyte is quite sensitive to the activating effect of glucagon whereas the human fat cell responds poorly (14) An in vivo lipolytic response to glucagon is clearly seen only in subjects with marked insulin deficiency

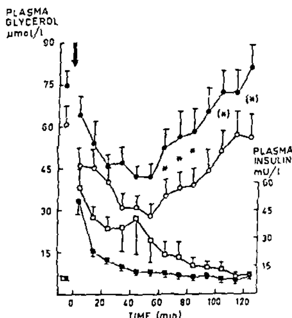


Fig 2 Effects of glucagon 0 5 mg i v (arrow) on plasma glycerol (● ○) and plasma insulin (■ □) in 8 low insulin responders (filled symbols) and 11 normals (open symbols) Mean ± S E Differences * $p<0.05$ (*) $0.05<p<0.10$

(11) Normal individuals in contrast tend to respond by a decrease in plasma glycerol this being attributed to glucagon induced insulin release (11)

Interpretation of glycerol data in the present study is difficult Although differences in insulin levels during the test were not statistically significant it is possible that the lesser degree of glycerol decline in low insulin responders was a consequence of a lower insulin stimulus on adipose tissue Furthermore since pharmacological amounts of glucagon may cause catecholamine release a difference between the groups in this regard may have influenced lipolysis rates In addition the groups may possibly have differed in adipocyte responsiveness to one or more of the hormones Finally glycerol levels are influenced by the rate of glycerol removal It is not known whether any difference in this process exists between the groups studied

Few data are available on the relation between glucose induced and glucagon induced insulin secretion In accordance with a recent study (17) we found no correlation between the results of these two insulin secretion tests

Although the present pharmacologic dose of glucagon did disclose one slight difference between the groups a similar test on a lower dose level may conceivably uncover other patterns of difference Experiments in progress aim at defining a suitably low glucagon dose for further comparisons between normals and low insulin responders

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Short-Term Effects of 1-Alpha-Hydroxy-Vitamin D_3 in Patients on Corticosteroid Treatment and in Patients with Senile Osteoporosis

Urban Lindgren Sam Lindholm and Bert Sarby

From the Departments of Orthopaedic Surgery and Radiation Physics Huddinge University Hospital Karolinska Institute Stockholm Sweden

ABSTRACT Six patients with bronchial asthma undergoing long term corticosteroid treatment and six patients with senile osteoporosis were given the same oral dose of 1 α hydroxy vitamin D_3 (1α OH D_3) and calcium The immediate effect on blood and urine chemistry and on the intestinal calcium absorption rate were studied Hypercalcaemia occurred frequently among the patients treated with corticosteroids but not among those with senile osteoporosis We conclude that corticosteroids do not counteract the effects of 1α-OH D_3 No correlation was found between the calcium absorption rate and the degree of osteoporosis nor did the serum PTH levels show any differences that could be attributed to the treatment

It has been shown that glucocorticosteroid treatment may lead to impaired intestinal calcium absorption (6 9 10) and to increased resorption of bone (6 8 14) Increased parathyroid activity secondary to the serum calcium lowering effect of corticosteroids might be responsible for the development of osteoporosis (1 7) The calcium lowering effect of corticosteroids has been interpreted as an antagonism between these and vitamin D most obvious with regard to the intestinal absorption of calcium In osteoporosis of old age an impaired calcium absorption mechanism is also apparent (4)

Earlier preliminary studies have indicated that 1-α hydroxy vitamin D_3 (1α OH D_3) has a positive effect on gerontoid osteoporosis (11) 1α OH D_3 which normally seems to be rapidly converted to $1,25(OH)_2D_3$ (13) has a positive effect on the intestinal absorption of calcium and on the mineralization of bone and might therefore also be used in the treatment of corticosteroid osteoporosis

The present investigation was undertaken to study the immediate effect of standard doses of 1α OH D_3 and Ca in patients undergoing corticosteroid treatment as compared with patients with senile osteoporosis

PATIENTS AND METHODS

One experimental group comprised six patients (3 females 3 males) with bronchial asthma treated with corticosteroids in doses equivalent to 5–10 mg of prednisolone for more than five years Their mean age was 64 years (range 52–74)

The other group consisted of six patients (3 females 3 males) with senile osteoporosis who had been treated for fracture of the femoral neck or for vertebral body fracture caused by minor trauma Their mean age was 75 years (range 70–84)

The following investigations were performed before and after treatment with a daily dose of 2 μg of 1α OH D_3 and 1 g of Ca for seven days *Calcium absorption tests* were carried out using an oral dose of 5 μCi of ^{45}Ca in 20 mg of calcium chloride dissolved in 250 ml of water Blood samples were collected at 15 30 45 60 90 and 120 min after the isotope administration The fraction of the administered activity circulating at the various times was determined by means of a liquid scintillation counter These values were fitted to the equation for the two-compartment model described by Marshall and Nordin (12) with the aid of a computer program which also calculated the absorption rate of calcium (α) expressed as a fraction of the given activity absorbed per hour (Fig 1) *Total serum calcium levels* were determined by atomic absorption spectrometry and *phosphorus and alkaline phosphatase levels* by spectrophotometric methods *The parathyroid hormone (PTH) level* in serum was measured by radioimmunoassay

The degree of osteoporosis was determined by (*a*) photon absorptiometry of the distal radius using ^{125}I and ^{241}Am as radiation sources (*b*) Singh s index (15) and (*c*) the vertebral and the combined peripheral scores (3) On these grounds osteoporosis was graded either slight or marked Osteoporosis was considered marked if at least two of the following criteria were fulfilled Densitometry

Table I *Effect of 1α-OH D_3 in patients with senile osteoporosis and in patients on corticosteroid treatment (mean ± S D)*

	Intestine		Serum		Urine	
	Ca absorption rate (α)	Ca (mmol/l)	P (mmol/l)	Alkaline phosphatase (μkat/l)	Ca (mmol/l)	P (mmol/l)
Senile group						
Untreated	0 57±0 19	2 36±0 16	1 19±0 19	3 85±1 23	2 31±1 20	20 50±9 24
1α OH D_3	0 94±0 19*	2 35±0 11	1 24±0 22	4 11±4 06	8 05±2 81*	22 75±3 30
Corticosteroid group						
Corticosteroid	0 77±0 44	2 32±0 19	1 07±0 45	2 83±0 36	6 15±3 30	22 83±8 4
Corticosteroid+ 1α OH D_3	1 09±0 60*	2 53±0 21*	1 16±0 32	3 18±1 05	8 05±3 50*	28 33±4 8*

*$p<0$ 05

<1 5 g/cm vertebral score <75 Singh s index <4 and *peripheral score <80 Those who did not meet with these* criteria were considered to have slight osteoporosis

Statistical evaluation was made using Student s *t* test for paired observations

RESULTS

In the group of *patients with senile osteoporosis* the intestinal calcium absorption rate rose significantly following treatment with 1α OH D_3 and Ca and so did the urinary excretion of calcium The serum lcium increased in some patients but there was no *rease for the group as a whole The levels of* rum and urinary phosphorus were insignificantly raised as was the level of alkaline phosphatase in *serum (Table I Fig 2)*

In the group of *patients undergoing corticosteroid treatment* a significant increase was noted not only in the intestinal absorption of calcium but also in serum calcium and in the excretion of calcium and phosphorus in urine (Table I Fig 2) Three patients in this group developed

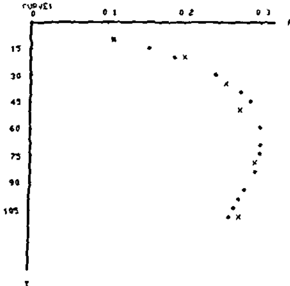


Fig 1 Example of computer printout with calculated α and β values The curve indicates fraction of administered activity (*F*) on horizontal axis related to time (*T*) in minutes after the appearance (T_0) of activity in serum (x=measured values *=fitted values) T_0 thus the time after isotope administration is extrapolated from the raw data on the activity measurements (Y_1–Y_6) by drawing a tangent through the steepest part of the rise of the time–activity curve Y_0 is a standard value related to the amount of given activity

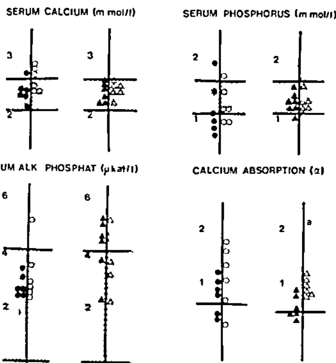


Fig 2 Immediate effect of 2 µg of 1α-OH D_3 and 1 g of Ca daily on calcium phosphate and alkaline phosphatase in serum and on intestinal calcium absorption ● = Corticosteroid treatment O = corticosteroid + 1α OH D_3 treatment ▲ = senile osteoporosis untreated Δ = senile osteoporosis treated with 1α OH D_3

hypercalcaemia Low serum phosphorus values were common in the patients of this group The intestinal absorption of calcium rose to very high values in some patients in the corticosteroid treated group One patient who did not show any increase in absorption rate had symptoms of acute gastroenteritis the night before the second test and this probably interfered with the result of the test

No correlation was found between the degree of osteoporosis whether calculated from the combined indices or from the densitometric values and the rate of calcium absorption Only minute changes in the serum PTH levels were found (Table II)

DISCUSSION

Since calcium absorption rate is known to be influenced by age (2) and like bone mineral content also shows considerable individual variations definite conclusions cannot be drawn from the present investigation However corticosteroid treatment in the doses used here did not seem to affect the calcium absorption rate to a major degree nor did the effects of 1α OH D_3 on calcium metabolism seem to be counteracted by the corticosteroids On the contrary many patients undergoing corticosteroid treatment had high calcium absorption rates and serum calcium levels Carre et al (5) found that rats treated with prednisolone had a high rate of degradation of 1 25$(OH)_2D_3$ in the intestinal mucosa the immediate effect of 1 25$(OH)_2D_3$ was however adequate which is in accordance with the

Table II *Degree of osteoporosis and effects of treatment with 1α-OH D_3 on serum PTH*

+ = Marked − = slight

Serum PTH (pmolEq/l)		Degree of osteoporosis	Densitometry (g/cm)
Untreated	Treated		
Senile group			
230	190	−	0 91
130	210	+	0 44
170	240	+	0 59
240	210	−	2 4
220	190	−	1 7
210	170	−	1 99
Corticosteroid group			
150	220	−	1 15
190	280	+	0 26
230	170	+	0 85
240	180	−	0 99
270	190	−	1 59
220	310	+	0 93

present findings in man An exaggerated response to 1α OH D_3 could for example be explained if a compensatory mechanism had developed opposing an impaired active Ca absorption system resulting from long term corticosteroid treatment In fact earlier findings by Kimberg et al (10) indicated the development of such a mechanism in the rat as a result of corticosteroid treatment

The present results suggest that 1α OH D_3 can be given to corticosteroid treated patients but if by assumption the effect of the drug on bone is indirect and slightly raised serum calcium and serum phosphorus levels are required a carefully adjusted individual dose for each patient is necessary

ACKNOWLEDGEMENTS

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Mild Phosphate Diabetes in Adults

Erik Lundberg Harald Bergengren and Bengt Lindqvist

From the Departments of Rheumatology Pathology and Medicine Umea University Umeå Sweden

ABSTRACT Phosphate diabetes has been considered as rare and to occur almost exclusively in children Upon examination of adult patients with rheumatic or kidney diseases it has, however been found that the combination of hypophosphataemia and hyperphosphaturia is not so rare This paper deals with 24 adult patients of this type, whom we have found during 6 months Their mean serum phosphorus concentration was 0 7 mmol/l (range 0 5–0 8) Mean phosphate clearance was 31 ml/min/1 73 m^2 (range 16–51) The diagnoses were myalgia, dorsalgia ($n=7$), papillitis calcificans ($n=5$) prostatitis or prostate accretions ($n=4$) dizziness ($n=2$), kidney stones, tubular defect interstitial nephritis medullary sponge kidney (1 case each), two patients had transplanted kidneys Asthenia was a common additional diagnosis The patients' complaints have been pain in the muscles, joints bones (18 cases) tiredness (10 cases), dizziness (8 cases), shakyness numbness, burning sensation (7 cases) tenderness in the muscles and bones ("the princess-on the pea syndrome) (7 cases) The most common findings upon examination were bone tenderness (13 cases) reduced manual power (8 cases) positive Romberg test (3 cases) slight muscle atrophy (2 cases), waddling gait (2 cases) The most common findings encountered in the laboratory, besides hypophosphataemia and hyperphosphaturia, were high pH in the urine, hyperaminoaciduria, and phosphate crystals in dried urine

Phosphate diabetes can be defined as hyperphosphaturia *in spite of hypophosphataemia* with a normal urinary calcium level and normal serum concentrations of calcium and parathyroid hormone The condition has been described in cases of primary renal hyperphosphaturia vitamin D resistant rachitis distal renal tubular acidosis and other tubular defects after kidney transplantation in association with various bone tumours so-called sclerotising bone angiopericytoma and during cortisone treatment of chronic joint diseases (3 5 9 13 16) Calcitonin has been shown like parathyroid hormone (1) to induce hyperphosphaturia The excretion of phosphates in the urine is reduced by 1 25-dihydroxy vitamin D_3 and 1 α hydroxy vitamin D_3 (12)

Phosphate diabetes has been diagnosed considerably more often in children than in adults Falkson and Frame (5) found 150 cases described in the literature but only 8% were adults Adult phosphate diabetes has also been described by Nagant and Krane (14) Phosphate diabetes gives rise to osteomalacia in children (5)

In adults severe phosphate diabetes can likewise give rise to osteomalacia (6 17) In milder cases of earlier stages the patients complaints are considerably more vague We have not found any general review of the patients difficulties in mild case of phosphate diabetes Case histories record complaints as bone tenderness pain in the bones back pain moderate muscle weakness pain in the soles of the feet diarrhoea and thin urine The patient s general condition is usually good Mild phosphate diabetes is probably a common condition We have not found any information in the literature concerning its frequency However this will depend on how phosphate diabetes is defined that is to say on the values for hyperphosphaturia and hypophosphataemia that are considered to permit such a diagnosis Phosphate diabetes can have clinical significance only if the patient is troubled by the condition

Over a period of six months we have met 24 patients with mild phosphate diabetes in the Departments of Rheumatology and Nephrology Treatment of the condition has attracted increased interest in that one now has access to less irritating phosphate tablets than previously as well as new active vitamin D preparations

METHOD

Phosphate level was estimated according to the ACU Chem method a modification of the Certific Chem method (4) For the diagnosis of hypophosphataemia a serum concentration of ≤0 8 mmol/l has been established and for the diagnosis of hyperphosphaturia a urinary excretion of at least 25 mmol/24 hours or a phosphate clear

Table I *Diagnoses complaints and findings at examination in the 24 patients with mild phosphate diabetes*

	No of pats
Diagnoses	
Myalgia dorsalgia or arthralgia	7
Papillitis calcificans	4
Prostatitis or prostate stone(s)	4
Transplanted kidney	2
Vertigo	2
Kidney stones	1
Tubular defect	1
Interstitial nephritis	1
Medullary sponge kidney	1
Complaints	
Pain in the muscles joints bones	18
Tiredness	10
Dizziness	8
Shakyness numbness burning sensation	7
Tenderness in muscles and bones	7
Feeling of pressure on the bladder	6
Findings	
Calf tenderness	13
Reduced manual power	8
Slight dizziness with Romberg s test	3
Slight muscle atrophy	2
Waddling gait	2

ance in excess of 15 ml/min/1 73 m² BSA We have not [illegible] dietetic restrictions in regard to phosphate rich [illegible] as we wanted to detect individuals with hyperphosphaturia and hypophosphataemia. With a phosphate-poor diet the frequency of hyperphosphaturia of course decreases while the frequency of hypophosphataemia increases Our main interest has been to ascertain how many patients on a normal diet have a phosphate-rich urinary excretion in spite of a low phosphate level in serum Urinary phosphate excretion investigations have been discussed by among others Nordin and Fraser (15)

Manual power has been measured with a balloon dynamometer Calf tenderness has been determined by a blood pressure cuff placed round the thickest part of the calf and slowly inflated the patient indicating when he/she begins to feel pain Normal values are 170–180 mmHg (10)

STUDY POPULATION

We have investigated several hundred patients mostly outpatients in the Departments of Rheumatology and Nephrology and have found 24 patients 14 men and 10 women with mild phosphate diabetes Their average age was 47 years (range 31–56) The mean serum phosphorus concentration was 0 7 mmol/l (range 0.5–0 8) Mean phosphate clearance was 31 ml/min/1 73 m² (range 16–51)

Treatment consisted in 21 cases of phosphate tablets (containing a mixture of Na–K–Mg–Ca-phosphate) in daily doses of 2–4 g (2) plus in 8 cases of 1-α-hydroxy vitamin D_3 0.5–1 0 μg daily (supplied by Lowens Pharmaceutical Copenhagen Denmark)

RESULTS

In most cases the onset was unclear—the condition had existed for many years prior to diagnosis The diagnoses are presented in Table I The patients complaints varied according to the basic illness (Table I) Three patients two of whom had undergone kidney transplantation had no or minimal trouble despite lack of phosphate The most usual findings upon examination are also listed in Table I Typical phosphate crystals can be seen in dried urine under the microscope (Fig 1) The urine pH was usually 6 4–7 4

Six typical cases of phosphate diabetes

Case 1 Female 47 years Long-standing migraine intermittent swellings pain in the loin and flank Sometimes also tiredness dizziness tenderness in bones and muscles Serum phosphate 0 6 mmol/l phosphate clearance 41 ml/min Abundance of phosphate crystals in dried urine Slight bone tenderness Treated with phosphate and 1-α vitamin D_3 Her troubles diminished

Case 2 Female 39 years Urgency of micturition for many years feeling of pressure on the bladder During the last year back pain tiredness Serum phosphate 0 4–0 8 mmol/l phosphate clearance 36 ml/min Good manual capacity no bone tenderness Abundant phosphate crystals in dried urine Her complaints disappeared after 4 months of phosphate treatment.

Case 3 Male 50 years Long-standing back pain increasing pain in all joints muscle cramp walking difficulty swaying walk Fatigue dizziness pressure on the chest In 1969 papillitis calcificans with mild azotaemia Serum creatinine level at present 240 μmol/l Aminoaciduria Serum phosphate 0 7 mmol/l phosphate clearance 33 ml/min Manual capacity somewhat reduced pain threshold in the calves at 80 mmHg low Treated with phosphate tablets and 1-α vitamin D_3 His troubles have diminished somewhat

Case 4 Female 49 years Long standing pain above the bladder in the lumbar region occasional headache During the last year attacks of dizziness sickness cramp in the hands loss of sensation in the fingers Upon examination nystagmus was found during the attacks Permanently tender legs Pain in the calves at 70 mmHg low Good manual capacity Serum phosphate 0 6–8 urinary phosphate 25 mmol/24 hours Treated with phosphate tablets for more than a year occasionally also with 1-α vitamin D_3 Serum phosphate normal Considerably improved

Case 5 Male 54 years Long standing complaint of pain in all parts of the body stomach acidity vomiting trembling sweating staggering uncertain gait Mild papil-

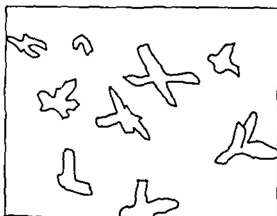


Fig 1 Triple phosphate crystals in dried urine

litis calcificans upon urography Serum phosphate 0 5–0 9 mmol/l Phosphate clearance 40 ml/min Mild aminoaciduria Mild liver disturbance Eosinophiluria Treatment with phosphate tablets and 1-α vitamin D_3 discontinued after a few days for fear of complications

Case 6 Female 47 years During the last 4 years pain in joints muscles intermittent small bleedings in the skin Electromyography showed myopathy Electron microscopy of muscle showed more glycogen than normal Examination indicated muscle tenderness muscle atrophy in the shoulder region Serum phosphate 0 7–0 9 mmol/l Urinary phosphate 25 mmol/24 hours phosphate clearance 21 ml/min Treated with phosphate tablets Improved

The short term effect of the treatment appears to be satisfactory but not good while the long term effect has not yet been established The results of treatment will be presented in another communication

None of the patients have had skeletal changes of the osteomalacia type upon X ray or osteoporosis with accompanying vertebral compression Only one patient had temporary insignificant hypercalcaemia hypercalcuria and/or raised parathyroid hormone concentration in the blood Aminoaciduria above normal was found in 5 of the 10 patients examined They had a loss of chiefly glycine and glutamine but also other amino acids were sometimes excreted in increased amounts

DISCUSSION

Bergengren (2) has described the manifestations of phosphate deficiency in cows with a high milk yield Phosphate deficiency arises from a lack of phosphate in the feed combined with phosphate losses via the milk The cows muscles became weak they had difficulty in walking due to pain in the joints and had tender bones (milk lameness) It has long been known that phosphate deficiency in man can lead to anorexia muscle weakness and bone tenderness (7 11) The condition has however been regarded as rare Essentially only children with nutritional insufficiencies or occasionally a child with tubular defect were supposed to be affected

It appears to us however that the condition has been seriously neglected Phosphate deficiency seems to be fairly frequent among adults Even mild cases of phosphate diabetes seem to give rise to long term complaints if the condition is disregarded for several years The troubles which afflict patients with slight phosphate diabetes are similar to those found both in animals with nutritional phosphate deficiency and in humans with severe phosphate diabetes

Patients with pain all over the body shifting from one spot to another from day to day (varialgia) are to be found in most practices Usually nothing is found upon examination The patients are assigned the diagnosis of asthenia neurasthenia myalgia varialgia arthralgia etc which make them misunderstood and unsatisfactorily treated In this group of asthenics there are certainly many cases of psychosocial insufficiency but no doubt also unrecognized somatic illnesses The frequency of phosphate diabetes among asthenics is unclear

Lindqvist and Lundstrom (10) have described a battery of tests suitable for asthenia manual power pain threshold in the calf breath holding time and time for filling in 1 000 squares Among these tests it appears that calf pain was the most important in phosphate diabetes followed by the manual power test (8) Ability to hold the breath appears to be normal in most cases We are in the process of evaluating bite pressure as some patients with phosphate diabetes complain about tender teeth

The cause of phosphate loss in the urine can be either a tubular defect or a hormonal imbalance possibly both factors are causative in some cases Clinical signs of the illness may not appear unless the patient has a phosphate-deficient diet The consumption of considerable quantities of food rich in phosphates may compensate the phosphate losses to such an extent that no appreciable phosphate deficiency occurs Among our patients those with kidney stone are likely to have been advised to eat

calcium deficient foods Unfortunately however calcium deficient diets are also low on phosphate and can thus lead to phosphate deficiency (2) Many patients have in addition gastritis and consume large quantities of aluminium hydroxide to combat stomach acidity In this way phosphate is removed from the intestine and the risk of phosphate deficiency increases in cases of mild phosphate diabetes Finally one can suspect that phosphate losses only gradually attain clinical significance The body has large phosphate stores but at present it is not possible to estimate a given individual s phosphate reserve Serum phosphate concentration is in all probability a poor indicator of phosphate deficiency

As we lack a method for estimating 1 25 dihydroxy vitamin D_3 in serum it is not clear whether this is a deficient factor that may underly a large number of cases of phosphate diabetes Estimation of 25 OH vitamin D_3 has not yet been shown to be of any importance

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A Tetracycline-Based Histomorphometric Evaluation of Bone Resorption and Bone Turnover in Hyperthyroidism and Hyperparathyroidism

Leif Mosekilde and Flemming Melsen

From Medical Department III University Institute of Pathology Surgical Department I and Department of Clinical Chemistry Århus Amtssygehus Århus Denmark

ABSTRACT Increased bone resorption previously found in hyperthyroidism might be caused by a direct stimulating effect of thyroid hormone(s) on bone cells or by an increased sensitivity to circulating parathyroid hormone In order to disclose qualitative differences in the response of bone resorbing cells to excess parathyroid hormone and excess thyroid hormone(s), histomorphometric analysis of iliac crest biopsies was performed in 25 hyperparathyroid and 40 hyperthyroid patients after tetracycline double labelling The main target cells for parathyroid and thyroid hormones were different Parathyroid hormone stimulated osteocytic osteolysis and increased osteoclastic resorption surfaces equally in trabecular and cortical bone The osteoclastic resorption was inactive Thyroid hormone(s) had no effect on osteocytes but increased the osteoclastic resorption surfaces in trabecular and cortical bone, with a pronounced preponderance in cortical bone The osteoclastic resorption was active and followed by a significant loss of both cortical and trabecular bone The findings support the assumption that increased bone resorption in hyperthyroidism is caused by a direct stimulating effect of thyroid hormone(s)

Increased bone resorption has been reported in both hyperparathyroid and hyperthyroid patients (2 3 4 13 14 16 20) It has been demonstrated that thyroid hormone(s) stimulates bone resorption in vitro (19) in the absence of parathyroid hormone and that thyroxine feeding increases bone resorption in both normal and parathyroidectomized dogs (1) This indicates that thyroid hormone(s) per se has a stimulating effect on bone resorption Studies in animals (10) and in man (5) have shown however that excess thyroid hormone(s) sensitizes and deficient thyroid hormone(s) impairs the responsiveness of bone to exogenous parathyroid hormone The increased bone mineral mobilization in hyperthyroid patients (17) therefore may be induced either by a specific stimulating effect of thyroid hormone(s) on bone cells or by an increased sensitivity of bone cells to circulating parathyroid hormone

Bone resorption might be caused by osteoclastic resorption on the surfaces of cortical and cancellous bone or by osteocytic osteolysis The aim of the present study was to compare bone resorption induced in vivo either by excess thyroid hormone(s) or by excess parathyroid hormone in order to disclose qualitative differences in the target cell response

PATIENTS AND METHODS

The study comprised 25 consecutively admitted euthyroid patients with primary hyperparathyroidism 18 women aged 39–75 years (mean 58) and 7 men aged 29–74 years (mean 55) and 40 consecutively admitted untreated patients with hyperthyroidism 33 women aged 16–79 years (mean 44) and 7 men aged 29–57 years (mean 48) The diagnosis of primary hyperparathyroidism was based upon determination of serum concentrations of immunoreactive parathyroid hormone (6) and total calcium corrected for individual variations in the serum albumin concentration (S-calcium corrected) In all cases a parathyroid adenoma was later found by neck exploration The diagnosis of hyperthyroidism was based upon clinical symptoms and signs determination of serum thyroxine (Tetralute ^{125}I Reagent kit Ames) serum triiodothyronine uptake test (Thyopac 3 Radiochemical Center Amersham) and absolute ^{131}I iodine uptake

Fasting serum concentrations of total calcium (S calcium mmol/l) and phosphorus (S-phosphorus mmol/l)

DISCUSSION

The present investigation demonstrates profound *qualitative* differences both at tissue level and at cellular level between the bone changes in hyper parathyroid and hyperthyroid patients We have previously reported on increased serum levels and urinary excretions of calcium and phosphorus in 45 hyperthyroid patients (17) The changes demon strated in calcium-phosphorus metabolism were positively correlated to the degree of hyperthyroid ism The serum concentration of iPTH was found to be decreased and inversely correlated to serum calcium These biochemical findings are not in consistent with the proposed theory (5) that the in creased bone resorption is caused by an increased cellular sensitivity to circulating parathyroid hor mone This is also true for the positive correlations found in a previous study (18) between the degree of hyperthyroidism and the histomorphometric parameters of bone resorption (RS CAR) in hyper thyroid patients The qualitative differences in bone resorption and bone dynamics observed in the pres ent study between hyperparathyroid and hyper thyroid patients however support the assumption that the increased bone resorption in hyperthyroid ism is caused by a direct stimulating effect of thyroid hormone(s) on bone resorbing cells

The bone turnover (ATCS×CR) was slightly in creased in the hyperparathyroid and markedly in creased in the hyperthyroid patients This shows that an increased number of bone remodeling foci (Basic Multicellular Units (8)) is initiated in unit of time per unit of surface area in both patient groups This increased birth rate of bone remodeling centers might contribute to the increase in osteoclastic resorption surfaces found in both groups

	hyperpara-thyroidism	hyperthyroi-dism	
CR	↑	↑	
ATCS	↑	→	
RR	↑	→	(↑?)
POL	↑	→	
CAR/RS	→	↑	

Fig 1 Qualitative differences in bone remodeling in hyperparathyroid and hyperthyroid patients (↑=increased →=unchanged ↓=decreased) Abbreviations as in Table II

The RS were identified as scalloped interruptions in the lamellar structure with or without osteoclasts We do not distinguish between active and inactive RS because the osteoclasts are very mobile and move across the bone surface (15) The amount of bone removed in unit of time depends however

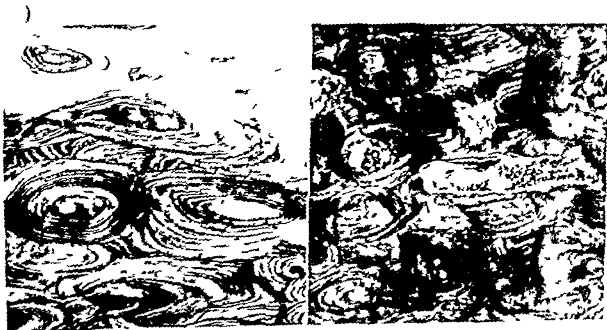


Fig 2 Cortical bone in hyperparathyroidism (to the left) and hyperthyroidism (to the right) Increased porosity and dilated canals with active resorption are found in cortical bone in the hyperthyroid patient (Polarized light magnification ×60)

not only on the extension of the resorption perimeter but also on the activity of the osteoclasts The linear RR cannot be measured but has to be estimated from changes in bone mineralization rate bone mass and RS (8) The decrease in the amount of trabecular bone with increasing age (12) will involve a slight underestimation of RR both in normal controls and in patients This net excess of resorption is however negligible compared with the normal turnover (0 7% and 10% of the amount of bone per year respectively (8))

In the present study we found a significant decrease in RR in the hyperparathyroid patients This inactive osteoclastic resorption in trabecular bone confirms previous suggestions (9) based upon studies on bone resorption in cortical bone The observation shows that osteoclasts stimulated by excess parathyroid hormone although numerous are less active than normal ones They are more numerous partly because of the increase in birth rate of new remodeling foci and partly because of a longer life span of each resorption focus (8) The normal amount of trabecular bone and the inactive osteoclastic resorption suggest that the increase in RS in primary hyperparathyroidism is of minor importance for the hypercalcemia. The positive correlation found between serum calcium and RS in this study and in others (16 20) need not indicate a causal interrelationship but might be a result of a covariation

RR was insignificantly increased in the hyperthyroid patients The decrease in AVTB demonstrates however that RR is underestimated It is possible to correct this error (8) knowing the mean duration of the hyperthyroid state (196±40 days) and assuming that the mean decrease in AVTB in this period equals the difference between AVTB in controls and in hyperthyroid patients and that the surface density of trabecular bone is normal (21 22) After this correction the mean linear RR was found to be 4 03 µm/day in the hyperthyroid patients

The increased RR in hyperthyroidism and the significant decrease in the amount of trabecular bone indicate that the osteoclastic resorption in trabecular bone might be of importance for the slight hypercalcemia although no correlations were found between RS and chemical indices of bone mineral mobilization The number of osteoclastic resorption lacunes are increased in hyperthyroidism mainly because of the marked increase in the birth rate of new remodeling foci since the life span of the osteoclasts probably is decreased (8)

The CAR was increased slightly in the hyperparathyroid and markedly in the hyperthyroid patients In both groups positive correlations were found between CAR and POR indicating that the increased CAR might be followed by increased porosity POR was however significantly increased only in the hyperthyroid patients The observed loss of cortical bone in hyperthyroidism and the positive correlations between CAR and biochemical indices of bone mineral mobilization indicate that the osteoclastic resorption is very active The positive correlations in the hyperparathyroid patients between CAR and urinary excretions of calcium and phosphorus suggest that the slight increase in CAR in this condition is of importance for the increased urinary excretions of calcium and phosphorus even though no decrease was noted in the amount of bone

The mean size of the POL was increased in the hyperparathyroid patients This supports previous investigations on the influence of parathyroid hormone on osteocytic osteolysis in patients with primary and secondary hyperparathyroidism (16) Furthermore POL is increased in animals after administration of parathyroid hormone (11) after EDTA infusions (16) and on low calcium diet (11) POL was slightly but insignificantly ($p<0.10$) increased in the hyperthyroid patients This might be explained by an abnormal representation of POL of different sizes in hyperthyroidism as previously discussed (18) The osteocytic osteolysis was in both patient groups unrelated to the chemical indices of bone mineral mobilization

The present investigation has shown that the main target cells are not the same for parathyroid and thyroid hormones in bone Parathyroid hormone stimulates the osteocytic osteolysis and increases the osteoclastic resorption surfaces equally in trabecular and cortical bone The osteoclastic resorption is rather inactive Thyroid hormone(s) has no effect on the osteocytes but increases the osteoclastic resorption surfaces in trabecular and cortical bone with a preponderance in cortical bone The osteoclastic resorption is active

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Serum Lipoproteins in Massive Obesity

A Study before and after Jejunoileal Shunt Operation

Stephan Rossner and Dag Hallberg

From the Department of Internal Medicine Karolinska Hospital the Department of Surgery Serafimerlasarettet and King Gustaf V Research Institute Stockholm Sweden

ABSTRACT Serum lipoproteins in 31 grossly obese males and females were determined by preparative ultracentrifugation before and after jejunoileal shunt operation The results were compared with data from a randomly selected group of healthy controls Before surgery a type IV hyperlipoproteinaemia was found in 5/11 men, which reverted to normal after operation The obese females had a lower VLDL TG concentration which did not fall after surgery In 10/11 males and 19/20 females the HDL cholesterol concentration was below the median value for controls before surgery The patients were followed up to 55 months after surgery and the lipoprotein pattern was repeatedly determined The mean weight loss was about 30% at the time of the last lipoprotein analysis The major finding was a marked reduction of LDL cholesterol, about 40% The LDL TG concentration was not affected The LDL composition thus changed at all TG concentrations the LDL of the operated patients contained less cholesterol than LDL of controls On agarose electrophoresis a β lipoprotein with increased mobility, referred to here as a "rapid β" lipoprotein, could be demonstrated in about 65% of the obese subjects before operation After surgery this "rapid β" was seen in about 80% of the cases No explanation for this lipoprotein abnormality can be offered as yet A negative correlation between HDL cholesterol and VLDL TG has been described in several earlier studies The values for our obese subjects were found below such a regression line of controls both before and after operation A low HDL cholesterol concentration seems to constitute a risk factor for the development of atherosclerotic manifestations Our data are in agreement with a hypothesis that the increased risks for atherosclerosis seen in obese subjects could be attributed in part to the low HDL cholesterol values seen in these subjects

An increased concentration of serum triglycerides (TG) is a common finding in obese individuals Even in moderately overweight subjects the incidence of hypertriglyceridaemia is high

Serum cholesterol may also be somewhat elevated in obese subjects, but this is not a consistent finding For a recent review see Nestel and Goldrick (10)

In a recent lipoprotein survey of healthy men and women a statistical analysis was carried out with and without overweight subjects (6) In that study overweight was defined as

$$\frac{\text{weight (kg)}}{\text{height (cm}-100)} > 1\ 10$$

Overweight healthy individuals were found to have higher total TG VLDL TG VLDL cholesterol and lower HDL cholesterol concentrations than non obese subjects

At the Department of Surgery subjects with massive obesity have been screened for possible jejunoileal shunt operation As part of the preoperative metabolic analysis serum lipid and lipoprotein concentrations were determined

It is well known that after jejunoileal shunt operation total serum cholesterol is markedly lowered (3) In a previous study we observed a reduction of 36% when the patients had become weight stable (14) However no significant change was found in the total serum TG concentration This suggests that the malabsorption induced by surgery influenced the composition of all or several lipoproteins The present study was undertaken to further analyze these preliminary observations

SUBJECTS

The subjects had all been referred to the Department of Surgery because of massive overweight which had resisted several conservative therapeutical methods The patients were at least 50% above normal body weight defined as a Broca index (kg/cm−100)=1 0 (Table I) Their overweight had lasted for more than five years and had led to severe social and psychiatric complications The patients included in the study were not on drugs known to affect lipid metabolism neither before nor after surgery Studies were not performed during any acute illness The bypass operation was either an end to-side

Table I *Clinical data and serum lipids and lipoproteins (mmol/l) (mean ± S E M) in grossly obese patients before jejunoileal shunt operation and lipid and lipoprotein values in non-obese controls (age adjusted to 40 y)*

	n	Age (y)	Body weight (kg)	Index (kg/cm − 100)	Total lipids		VLDL		LDL		HDL	
					TG	Chol	TG	Chol	TG	Chol	TG	Chol
Males												
Patients	11	39±3	150±5	1.82 ±0.07	2.62 ±0.33	5.87 ±0.33	1.86 ±0.30	0.88 ±0.12	0.53 ±0.06	3.83 ±0.26	0.20 ±0.02	1.06 ±0.12
Controls					1.80	6.40	1.00	0.52	0.52	4.20	0.24	1.37
Females												
Patients	20	42±2	127±4	2.03 ±0.06	1.61 ±0.12	5.33 ±0.20	0.90 ±0.10	0.41 ±0.05	0.43 ±0.03	3.65 ±0.20	0.21 ±0.02	1.13 ±0.05
Controls					1.40	6.71	0.67	0.34	0.48	4.17	0.27	1.79

anastomosis between jejunum and ileum or an end-to-end anastomosis (7). Of a total intestinal length of 5–8 m, 50–65 cm remained after the shunt. All patients were on a free diet before and after surgery. However, several patients spontaneously changed their dietary habits after the operation. This could certainly influence the lipoprotein composition but was not controlled in this study. All patients received a multivitamin preparation orally daily and 1 mg of vitamin B_{12} i.m. every second month.

Thirty-one grossly obese patients (11 males, 20 females) were studied in the preoperative screening. However, for various reasons, not all of them were subsequently operated. Postoperative values were obtained in 8 males and 23 females, some patients already having been operated when the present study was initiated. Values both before and after surgery were available in 7 males and 14 females.

METHODS

...separation

...asting specimen of venous blood was drawn without stasis and was left to clot at room temperature. The lipoprotein separation and analysis have been described in detail elsewhere (4). In summary, serum was prepared by slow speed centrifugation and 1% Na EDTA was added. By preparative ultracentrifugation at $d = 1.006$ the top fraction (VLDL) was isolated. In the infranatant the LDL fraction was then precipitated with $MnCl_2$-heparin. Thus the HDL fraction remained in solution. By means of a Technicon AutoAnalyzer Model I, TG and cholesterol concentrations were determined after isopropanol extraction in whole serum, the top (VLDL) fraction, the bottom (LDL + HDL) and the HDL fraction after precipitation (2, 8). The concentrations of TG and cholesterol in LDL were obtained after subtraction of the HDL values.

In this study a type IV hyperlipoproteinaemia, according to the WHO classification (1), was defined as a VLDL TG concentration above the 90th percentile of control values, absence of a significant late pre β lipoprotein (4) and a LDL cholesterol concentration below the 90th percentile of control values (13).

Agarose gel lipoprotein electrophoresis was performed on whole serum, top and bottom fractions after separation at $d = 1.006$ as described by Noble (11). Staining was done with Sudan black. The electrophoretic patterns were interpreted by several individuals independently.

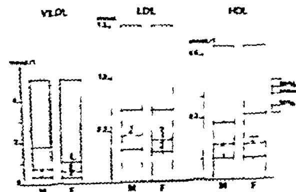


Fig 1. TG concentrations in VLDL, LDL and HDL in grossly obese males (*M*) and females (*F*) before shunt operation. Control values (ref 6) are given, showing the 10th and 90th percentile and the median value.

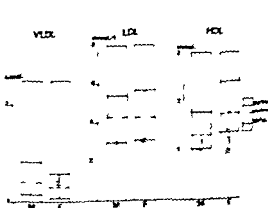


Fig 2. Cholesterol concentrations in VLDL, LDL and HDL. Symbols as in Fig. 1.

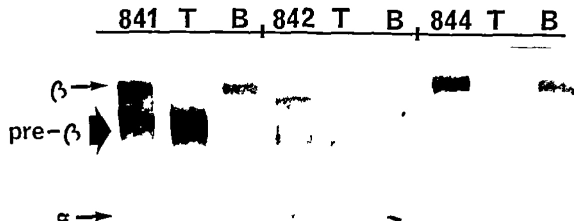


Fig 3 Agarose electrophoresis of lipoproteins from a grossly obese subject The electrophoresis was carried out on whole serum top (*T*) and bottom (*B*) fractions after ultracentrifugation at $d=1\ 006$ Two control samples are run in parallel The β lipoprotein of the obese sample (no 842) has markedly increased mobility the so-called rapid β'

CONTROL SUBJECTS

Lipoproteins

The data from the obese subjects were compared with those from a control group of non-obese men and women from Uppsala who had been carefully studied and declared healthy (6) Although TG and cholesterol concentrations in total serum VLDL and LDL increased with age linear regression analysis of control data revealed that only the increases in females were statistically significant For comparison lipoprotein figures adjusted to 40 years are given in Table I In this study the same lipoprotein isolation method had been applied but TG and cholesterol were determined on an AutoAnalyzer Model II (Technicon) which gave identical cholesterol values but 0 2 mmol/l higher values for total TG and LDL TG (Carlson unpublished data)

Rapid β' lipoprotein

At the time of the Uppsala study the rapid β' lipoprotein had not been systematically studied Control figures from a group of randomly selected 40-year-old men from Stockholm have recently shown that the rapid β' lipoprotein was observed in 9% of these subjects (the Stockholm–Edinburgh study unpublished data)

RESULTS

Before surgery

Clinical data and the mean values of lipoproteins for obese males and females before surgery as well as control values are summarized in Table I In Figs 1 and 2 the individual preoperative values are plotted together with the lipoprotein values for the control group In men mean serum TG values were increased before surgery This TG increase was due to elevated VLDL TG concentrations and a type IV lipoprotein pattern was found in 5/11 men The VLDL cholesterol concentration was also increased but in proportion to the VLDL TG so that the cholesterol/TG ratio in VLDL did not differ from the control value The cholesterol concentration in LDL did not differ from that of the control group In 10/11 males the HDL cholesterol was well below the median value for controls In women mean total TG concentration was lower than in men In VLDL a slight TG increase was found and VLDL cholesterol was increased in parallel Type IV pattern was found in 7 of 20 women LDL and HDL concentrations of TG in the obese women did not differ from the control values In 19/20 women the HDL cholesterol concentration was well below the median for controls

Agarose electrophoresis

On electrophoresis a β lipoprotein with increased mobility compared to control plasma samples was observed in 8/11 men and 12/20 women An example of such a rapid β' lipoprotein is shown in Fig 3

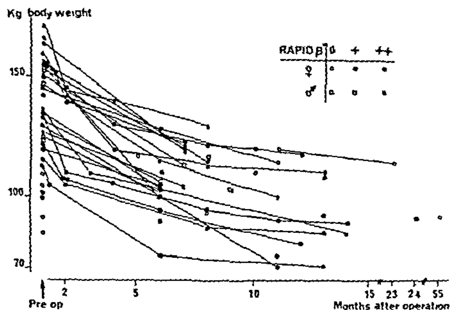


Fig 4 Weight reduction pattern in grossly obese subjects after shunt operation The appearance of a β-lipoprotein with increased migration velocity a so-called "rapid β" was evaluated by inspection The rapid β velocity was graded semiquantitatively and is indicated by half-closed or closed symbols

After surgery

The weight reduction pattern after surgery is demonstrated in Fig 4 In patients studied both before and after surgery the preoperative body weight in males was 150±6 (S E M) kg and in females 130±5 kg At the time of the last lipoprotein analysis after operation the weights were 110±6 kg in males and 100±4 kg in females The interval from surgery to the last lipoprotein analysis was 7–12 months in males and 2–55 months in females In Figs 5 and 6 a direct comparison is made in those ...ects who were studied both before and after ...nt operation The effects of the shunt operation ...serum lipoprotein can be summarized in the ...ollowing way

Lipoprotein concentrations

In men the total serum TG concentration was reduced This was due to a reduction in VLDL TG with a tendency for LDL TG to fall (Fig 5) The marked reduction in total serum cholesterol after shunt operation was mainly attributable to a 42% LDL cholesterol fall with a minor contribution from VLDL cholesterol The cholesterol/TG ratio in VLDL was not affected after the operation No type IV pattern was observed after surgery

In females the pattern was slightly different (Fig 6) The total TG concentration remained unchanged and the only significant changes in concentration within the lipoprotein classes was the marked reduction of the LDL cholesterol concentration

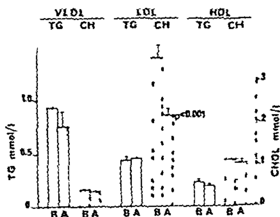


Fig 5 TG and cholesterol concentrations in VLDL, LDL and HDL in obese males ($n=7$) before (*B*) and after (*A*) shunt operation Significant change (Student's test for paired data) is indicated

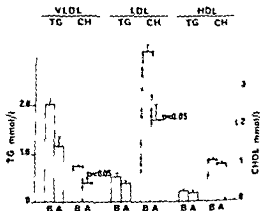


Fig 6 TG and cholesterol concentrations in VLDL LDL and HDL in obese females ($n=14$) Symbols as in Fig 5

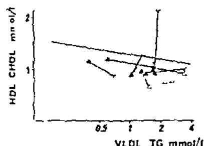


Fig 7 Relationship between VLDL TG and HDL cholesterol in obese men before (Δ) and after (▲) surgery The corresponding regression line for the control subjects is indicated Semilogarithmic scales

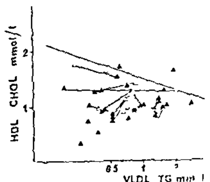


Fig 8 Relationship betw VLD cholesterol in obese femal s b f e (gery The corresponding reg es subjects is indicated Semi

(40%) Thus males and females showed similar changes in LDL cholesterol Since the VLDL TG concentration was not affected in females in contrast to males 9/27 females had type IV patterns after the operation

In the control subjects a negative relationship between VLDL TG and HDL cholesterol has been demonstrated previously (6) When the data from the obese patients were related to the corresponding regression lines for the control subjects 2 males were found above and 9 below the regression line before surgery After surgery all males were below this line (Fig 7) All 20 obese females studied before surgery had lower HDL cholesterol values than expected from their VLDL TG concentration and after surgery 25/27 females were found below the regression line (Fig 8)

The LDL composition fore and after surgery was data Fig 9 demonstrate 10/11 males were found a for controls (It should be k TG in controls was 0 2 mmol gery 7/8 male obese patients w This would indicate that for concentration after surgery the L tained more TG than expected pattern can be seen for females Before su LDL cholesterol concentrations in relation to LDL TG were found in 13/20 female patients whereas 23/27 operated females were found below the regression line for the control group

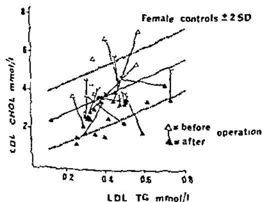


Fig 9 Relationship between LDL TG and cholesterol concentrations in male obese subjects before and after shunt operation The regression line ±2 S D for the control subjects indicated

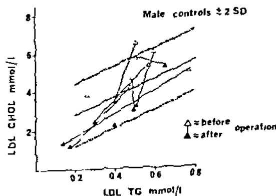


Fig 10 Relationship between LDL TG and cholesterol concentrations in female obese subjects before and after shunt operation The regression line ±2 S D for the control subjects is indicated

Agarose electrophoresis

On agarose electrophoresis a β lipoprotein with rapid mobility both in whole serum and in the bottom fraction as compared to parallel control samples was the most striking observation in the obese subjects and was observed already before surgery If this lipoprotein mobility pattern was present already before operation it generally persisted afterwards However some patients who did not exhibit the rapid β' pattern before surgery developed this during the postoperative weight losing period The weight reduction pattern and the time for the lipoprotein studies after surgery are given and the appearance of rapid β' is indicated in Fig 4 At the last postoperative study the rapid β' was found in 7/8 males and 22/27 females Another finding on agarose electrophoresis was the presence of a pre β lipoprotein which appeared in the top fraction with a reduced mobility the so-called late pre β lipoprotein (5) Before surgery this late pre β' pattern was seen in 2/11 males and 3/20 females After surgery the pattern was seen in 2/8 males and 7/28 females

Since many of the patients had abnormal LDL after operation it is conceivable that this lipoprotein had different properties during the precipitation with heparin $MnCl_2$ However control with agarose electrophoresis of the bottom fraction did not reveal coprecipitation or abnormal solubility

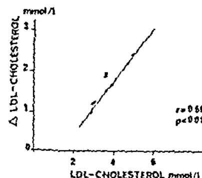


Fig 11 Relationship between *preoperative* LDL cholesterol concentration and absolute reduction of cholesterol after surgery in obese males and females

DISCUSSION

Our study confirms that in both males and females the increase in serum TG well documented in advanced obesity was due to increased VLDL TG concentrations whereas the TG concentrations in the other lipoprotein classes were within the limits of the control values

For cholesterol the situation was different Total serum cholesterol was well within the limits of the control values in both sexes However this was the result of an increase in VLDL cholesterol combined with a concomitant decrease in HDL cholesterol A negative correlation between the VLDL TG concentration and the HDL cholesterol concentration has been found in previous populations studied (6 9 13) Figs 7 and 8 demonstrate that in obesity the HDL cholesterol concentration was lower than expected for any given VLDL TG concentration in both sexes There is evidence that obesity constitutes a risk factor for the development of atherosclerotic manifestations (10) Furthermore a high HDL concentration seems to exert a protective effect against such manifestations (9) The results of our study are thus consistent with a hypothesis that obese subjects are more prone to atherosclerotic manifestations than controls because of among other possible factors their low HDL cholesterol and high VLDL concentrations

It is of interest that in males the VLDL TG concentration was reduced by about 50% after surgery but the HDL cholesterol concentration remained low Thus there seems to be a dissociation between the negative relation between these two parameters after operation HDL seems to have a role in the efficient removal of VLDL from the circulation (9) The fall in VLDL in obese men on the other hand seems to be caused by other factors than a high HDL concentration Our results underline the importance of lipoprotein analyses The determination of only total serum lipids would not have made it possible to detect the disturbance of the cholesterol distribution in these patients

The weight reductions after surgery followed the same general pattern as previously described (7) The major change in lipoprotein composition after operation was the marked reduction of LDL cholesterol in both sexes The higher the initial LDL cholesterol concentration the greater was the absolute reduction after surgery as shown in Fig 11 LDL TG were not affected in parallel and the resulting change in the composition of LDL particle is demonstrated in Figs 9 and 10 There was one sex difference in that both TG and cholesterol in VLDL fell in parallel in men whereas females who

had lower VLDL concentrations before surgery did not show this reduction

The significance of the rapid β lipoprotein found in 65% of the obese subjects before surgery is unclear The concentrations of TG and cholesterol in the lipoprotein fractions did not differ between patients with and without rapid β lipoprotein Several factors might influence the mobility of the β-lipoprotein such as increased plasma concentrations of free fatty acids or the apoprotein composition the electric charges the concentration or the size of the LDL particles

Preliminary studies in our laboratory have shown that the FFA concentration in plasma with the rapid β lipoprotein was not higher than in control plasma (unpublished results) Further studies of the LDL particle in rapid β' cases are in progress in order to determine the cause of this new lipoprotein abnormality The rapid β lipoprotein is however not specific for obesity In lipoprotein screening in our lipid clinic this lipoprotein has been found in several other conditions However a rapid β lipoprotein is seen in only about 5% of all lipoprotein electrophoresis analyses carried out in our laboratory and obesity seems to be one of the major clinical findings correlated to the rapid β' lipoprotein

After surgery the rapid β lipoprotein was seen in up to 80% of the obese patients The factors leading to this increase are not known at present The lipoprotein abnormality appears to be very constant in the obese patients since it persisted throughout the follow up period once it could be demonstrated The rapid β lipoprotein was found in almost all males both before and after surgery but in only about 50% of the females Since the concentrations of both TG and cholesterol in LDL were very much alike in both sexes the TG/cholesterol ratio cannot account for the sex differences

Shunt operations have been used to treat patients with advanced hyperlipoproteinaemia and marked reductions of serum lipoproteins have been reported (3) However in these patients a partial bypass has generally been made and the effect on body weight has been little or none Such results are not comparable with those obtained in our study In a group of obese patients submitted to end to-end jejunoileal bypass electrophoretograms were carried out before and after surgery and the lipoprotein patterns were classified according to the WHO typing system (15) In 27 of these patients a type IV pattern was found before surgery In all cases the type IV pattern normalized during the follow up period No analyses of TG and cholesterol in the lipoprotein classes were included in the above study but total serum lipids were determined and cholesterol fell from a mean value of 205 mg/100 ml by 45% and total TG from a mean value of 268 mg/100 ml by 53% These results were not given for each sex and a direct comparison with our results cannot be made

The dramatic weight loss induced by the jejunoileal shunt operation in grossly obese subjects mainly affects the LDL cholesterol concentration There is evidence that VLDL synthesis may occur in the intestinal wall (12 16) Our data do not rule out the possibility that the VLDL reduction is the result of impaired VLDL synthesis in the intestinal parts shunted away by the operation but the effects on VLDL TG could also be the result of the weight loss per se rather than be caused by the malabsorptive state induced by the shunt

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Serum Triglycerides and Cholesterol and Serum High-Density Lipoprotein Cholesterol in Highly Physically Active Men

A Lehtonen and J Viikari

From the Department of Internal Medicine University of Turku Turku Finland

ABSTRACT The influence of extensive physical activity upon plasma lipids, in particular HDL cholesterol, was investigated The material consisted of 23 regularly training men (mean age 44 years, average exercise 83 km running or skiing weekly), 15 healthy men (mean age 47 years), 10 young men (mean age 22 years), 12 healthy women (mean age 32 years) and 18 hyperlipidaemic patients The exercise increased serum HDL cholesterol and FFA concentrations and decreased triglyceride levels significantly, but had no significant effect upon serum cholesterol concentration There was a positive correlation between the amount of weekly exercise in km and plasma HDL cholesterol concentration Exercising more than 70 km/week increased plasma HDL concentration clearly above the normal level The advantages of an increase in plasma HDL cholesterol are discussed

Even mildly raised serum levels of cholesterol (25) and triglycerides (4) are considered to be risk factors of ischaemic heart disease Miller and Miller (17) postulated a negative association of serum high-density lipoprotein (HDL) and coronary heart disease (CHD) The possibility has been raised that high concentrations of HDL may afford some protection against CHD (2 5 20) The significance of decreased α cholesterol levels in CHD has been confirmed in prospective studies (15 22) The distribution of plasma lipoprotein cholesterol may be influenced by the level of habitual physical activity so that very active persons have higher HDL levels (6 8 13 27) Fasting plasma triglyceride concentrations have been found to be lower in physically well trained men than in their sedentary counterparts (9 10 27) An increased physical activity generally results in no or only a minor decrease in plasma total cholesterol level (9 11)

We have compared the concentrations of serum cholesterol and triglycerides and HDL cholesterol of 23 middle aged or older men undergoing regular physical training with those of selected control groups and correlated them with the amount of physical activity

SUBJECTS

Twenty three active men (group A) had trained regularly for at least five years This group was limited to men 30 years old or more who had trained four or more times weekly and had averaged at least 25 km/week running or skiing The control groups were 15 healthy men over 30 years without regular exercise (group B) 10 healthy young men with exercise less than 25 km/week (group C) 12 healthy women (group D) and 18 patients (7 females 11 males) with type II A hyperlipoproteinaemia (group E) The subjects completed a questionnaire on their normal diet smoking habits and alcohol habits

METHODS

Venous blood was drawn after the subjects had fasted overnight for 12–16 hours they were requested not to exercise during the fasting period Most subjects had been exercising during the previous day Plasma was separated by low speed centrifugation VLDL (very low-density lipoproteins) and LDL (low density lipoproteins) were precipitated with a polyethylene glycol solution (PEG 6000 12%) HDL was determined from the supernatant after removing the precipitate as described earlier (26) Cholesterol was measured by a modification of the method of Badzio and Boczon (1) Triglycerides were measured by the method of Carlson (3) Free fatty acids were determined according to Mikać Devic et al (16)

RESULTS

All runners had trained regularly for more than five years (6–50 years) most being life long runners Only five reported beginning regular running at the age of about 30 years or later The runners were all non smokers all had a normal diet except one with

Table I *Age and physique of runners and non active men*

	Age (y)	Weight (kg)	Height (cm)	Weight / height − 100
Group A				
Mean±S D	43 6±9 1	70 3±6 4	176 0±5 0	0 93±0 07
Range	35–68	59–80	166–185	
Group B				
Mean±S D	46 5±7 6	83 0±9 1	179 3±6 5	1 05±0 09
Range	33–58	69–101	166–188	
Group C				
Mean±S D	21 9±2 9	68 3±7 7	180 4±5 6	0 85±0 08
Range	19–29	62–85	170–188	

sugar limitation and their alcohol intake was low or moderate

The age weight and height of the athletes (group A) and the control groups of healthy men (groups B and C) are shown in Table I Groups B and C comprised 5 and 2 smokers respectively All had a normal diet

Plasma lipid concentrations in runners and control subjects are shown in Table II Fasting plasma triglyceride levels of the runners were significantly lower than those of the age matched controls (group B) Plasma total cholesterol concentrations of the runners were not significantly lower HDL cholesterol concentrations of runners were higher compared to other groups The results are shown in Table II and Fig 1 Healthy women had somewhat higher mean HDL cholesterol levels than men The subjects with hyperlipoproteinaemia had the lowest HDL cholesterol concentrations Hyperlipidaemic women had higher HDL cholesterol levels than hyperlipidaemic men

There was no significant difference in HDL cholesterol concentrations between groups B and C Fig 2 shows a positive correlation between the number of km run per week and the HDL cholesterol concentration The fatty acid concentration of the runners was significantly higher than that of the controls (group B)

DISCUSSION

The relationship between physical activity and serum lipid levels especially serum cholesterol has not been definitively established A program of vigorous exercise has been followed by a modest reduction of the serum cholesterol concentration (11 13 27) while in other studies no such change was observed (9) Our group of runners had trained

Table II *Plasma lipid and fatty acid concentrations in runners and control subjects (mean ±S D)*

Group	Total cholesterol (mg/100 ml)	HDL cholesterol (mg/100 ml)	HDL cholesterol / total cholesterol	Triglycerides (mmol/l)	Fatty acids (mmol/l)
A	190 5±38 9	68 5±14 9	0 37±0 08	0 59±0 27	0 78±0 25
B	201 6±47 1ns	54 9±12 1**	0 29±0 08 *	0 92±0 41**	0 51±0 15
C	174 1±47 2ns	56 3±10 8*	0 33±0 07ns	0 66±0 15ns	
D	218 9±33 7	59 8±9 1	0 28±0 07		
E Males	312 7±67 4	44 2±9 8	0 14±0 03	1 70±0 96	
Females	334 1±120 4	54 0±17 6	0 17±0 06	1 41±0 67	

Significance of differences between groups A and B and A and C *$p<0.05$ **$p<0.01$ ***$p<0.001$ ns=non significant

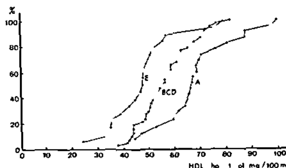


Fig 1 Cumulative distribution of HDL cholesterol *A*=exercise group (*n* 23) *B C D*=combined control group (*n*=37) *E*=hyperlipidaemic patients (*n*=18)

almost continuously for several years This high grade physical training may be the cause of the relatively low total cholesterol values of the runners The finding of a low concentration of plasma triglycerides in runners is consistent with many earlier reports (9 10 27) Our results also agree with an earlier report (10) that plasma triglyceride concentration and age are positively correlated in sedentary subjects but not in athletes Our group of runners expended an average of about 5000 kcal/week more energy than the physically inactive controls Their diet was liberal (one subject had a sugar limitation diet) and they were not overweight Our results are not consistent with those of Mann et al (14) who reported an increase in plasma triglyceride concentrations after 6 months of moderate exercise and attributed the rise to greater food consumption The runner s low degree of adiposity may be responsible for part of the decreased triglyceride concentration since a positive correlation has been reported between relative weight and plasma triglycerides (24)

The high HDL cholesterol levels in the runners are very interesting Our results agree with those of Wood et al (27) who found higher mean levels of HDL cholesterol in runners than in a comparison group We found a positive correlation between the number of km run per week and the plasma HDL cholesterol concentration Our subjects in group A had all trained for several years most were life long runners and the amount of physical activity was high they had top-level physical fitness in their age class Our results very strongly support the idea that the effect of increased physical activity on plasma lipoproteins is advantageous However these findings are prominent only after a considerable amount of regular vigorous exercise Although there was a tendency to a higher than average HDL cholesterol concentration in the whole group of runners the concentrations were clearly higher than normal only when the amount of running exceeded 70 km/week This high exercise requirement agrees with an earlier study (18) where no relation was found between HDL cholesterol and physical activity Results of dietary questionnaires of the runners and control groups suggested that ethanol and food intake habits were similar in all groups The runner's lower level of adiposity cannot be considered responsible for part of the increased HDL cholesterol levels in runners compared with control men of the same age because the HDL cholesterol concentration of the younger men with low levels of adiposity was similar to that of the older men A higher plasma fatty acid concentration of the runners suggests an increased degree of adipose tissue lipolysis which is also reported in an earlier study (10) although there the plasma fatty acid concentrations were not higher in the physically active group than in the sedentary subjects Many other reports have supported the view that relatively low levels of plasma HDL cholesterol are associated with an increased risk of CHD (2 5 7 12 20)

Hyperlipidaemia is a common finding in patients with ischaemic heart disease (12 23) and prospective studies have shown an increased incidence of ischaemic heart disease with even mildly raised serum levels of cholesterol (25) and triglycerides (4) There is however an inverse relationship between VLDL triglycerides and HDL cholesterol as summarized by Miller and Miller (17) so that in fact

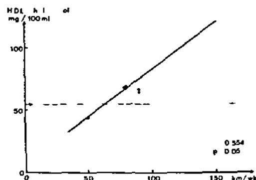


Fig 2 Correlation of HDL cholesterol to the amount of weekly exercise (group A) ---=Mean level of the age matched controls (group B)

the decrease in serum triglycerides and the increase in serum HDL cholesterol may reflect the same phenomenon The changes in plasma lipid patterns of the runners may protect against the development of CHD and the results support reports that vigorous exercise (19) and work acitvity (21) decrease the incidence of CHD and coronary mortality

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Hypergonadotropic Hypogonadism in Oligophrenia

P O Lundberg A C Nilsson and L Wide

From the Departments of Neurology and Clinical Chemistry University Hospital Uppsala Sweden

ABSTRACT Eight patients representing five different, probably hereditary neurological syndromes with oligophrenia and hypogonadism as the common features have been examined clinically and endocrinologically Two sisters suffered from polyneuropathy, one male from ataxia one male from spastic tetraplegia, two sisters and a brother from myopathy and one male patient from epilepsy and polyneuropathy The latter patient was diagnosed as having an acute intermittent porphyria *All the patients had degenerative neurological dis*orders The karyotypes were normal The patients all had signs of hypogonadism Four male patients had marked testicular atrophy but otherwise normal external genitalia The testosterone levels in the blood were normal or slightly decreased Three of the females had their menarche at a normal age but a very early menopause The fourth female has never menstruated The four females had normal breasts and body hair All patients had high basal luteinizing hormone (LH) and follicle stimulating hormone levels and the response to i v LH releasing hormone was exaggerated The prolactin values were normal None of the examined patients had any signs of thyroid or adrenal insufficiency and the sella turcica was normal A possible etiology to their hypergonadotropic hypogonadism is discussed

In patients with cerebral disorders of different kinds endocrine insufficiency including hypogonadism is not uncommon In most cases this is explainable by the fact that the brain lesion also includes damage to the hypothalamus thus leading to a disturbed hypothalamo-pituitary gonadal regulation Such patients usually have other symptoms and signs of hypothalamo-pituitary insufficiency Pituitary hormones including follicle stimulating hormone (FSH) and luteinizing hormone (LH) are decreased or within the normal range

However a hypergonadotropic hypogonadism is occasionally seen in patients with neurological disorders Some of these patients are cases of Klinefelter s or Turner s syndrome but genetically induced combinations of hypergonadotropic hypogonadism and different neurological symptoms including oligophrenia are also known The mechanism of the hypogonadism in the latter cases is unknown

Our systematic studies of endocrine symptoms in a large number of patients with oligophrenia have revealed hypergonadotropic hypogonadism in eight cases all with a normal karyotype These patients who probably represent five different hereditary syndromes will be presented and a possible etiology to their hypergonadotropic hypogonadism will be discussed

METHODS

Prolactin FSH LH oestradiol and testosterone in serum were determined by radioimmunological techniques Prolactin in serum was measured by a radioimmunosorbent technique using rabbit antihuman prolactin antibodies coupled to CNBr activated Sephadex (46 47) Prolactin and antiprolactin preparations were supplied by NIAMDD National Institutes of Health Bethesda USA FSH and LH in serum were determined by a radioimmunosorbent assay with indirectly coupled antibodies (48)

The results were expressed in $\mu g/l$ of highly purified human pituitary FSH and LH preparations (31 32) Oestradiol-17β in serum was determined by a radioimmunoassay using an antiserum to oestradiol-6-oxime BSA (16) and testosterone with an antiserum to testosterone 3-oxime BSA (6) Tests with luteinizing hormone–releasing hormone (LH RH) were performed as previously described (21 22) Reference values for prolactin FSH LH oestradiol and testosterone are given in Table I

SUBJECTS

The eight patients seem to represent five different syndromes Besides oligophrenia and hypogonadism all had neurological symptoms but of different types In two sisters (cases 1 and 2) a polyneuropathy was the main neurological symptom in case 3 cerebellar atrophy with ataxia in case 4 spastic quadriparesis in three siblings (cases 5–7) myopathy was the main cause of the neurological deficit and case 8 had epilepsy and polyneuropathy Case 8 was also found to suffer from acute intermittent

Table I *Normal values for prolactin follicle stimulating hormone (FSH) luteinizing hormone (LH) oestradiol and testosterone*

		Women	
	Men	Premeno-pausal	Postmeno-pausal
Prolactin (µg/l)	<15	<15	<15
FSH (µg/l)	0 5–3 0	0 5–3 0	5–25
LH (µg/l)	0 4–3 0	0 4–3 0	2–10
Oestradiol (pmol/l)	70–165	90–1 100	70–165
Testosterone (nmol/l)	9–29	1–4	1–4

ᵃ Excluding mid-cyclic LH peak

porphyria The five syndromes are difficult to classify but two of them have been described as new syndromes (19 20) Four of the syndromes seem to be hereditary whereas case 3 has no known relatives with similar symptoms

Oligophrenia and polyneuropathy

A new syndrome called hereditary polyneuropathy oligophrenia premature menopause and acromicria described by Lundberg (19)

Case 1 A woman 63 years of age whose parents had the same mother She had two younger sisters with similar symptoms One is described as case 2 The other one died many years ago According to case records her menstrual ods ceased when she was 26 years old The patient ed to walk and talk within the normal time Because difficulties in learning to read she was transferred to a cial school at 11 years of age Since then she has lived in institutions for the mentally retarded Her menarche occurred at 12 years of age She had normal menstrual periods until she was 25 years old and since then amenorrhoea She has walked unsteadily as long as she can remember Her neurological symptoms have slowly progressed At 62 years of age she was no longer able to walk without support

When examined at that age she was small and obese She had normal female body hair distribution Her breasts were normal but the nipples were pale and atrophic She had pronounced atrophy and pareses of all small muscles of the hands and feet All tendon reflexes in the extremities were absent The sensation in her hands and feet was impaired

According to earlier psychometric examinations she is considered to lie at a low debility level EMG showed a pattern compatible with a marked peripheral neurogenic lesion The motor nerve conduction velocity in the ulnar nerves was 25–28 m/sec Pelvic X rays showed a small pelvic skeleton X rays of the shoulders knees and bones of the hands and feet showed that the skeleton generally was abnormally small

Case 2 A woman 57 years of age sister of case 1 She had difficulties in managing school work but learned to read and write At the age of 7 years she began to develop weakness of her legs and gradually her hands began to lose strength Her menarche came at 13 years Her menstrual periods were irregular and terminated between 18 and 20 years of age

The patient was examined at 44 years of age She was small and pronouncedly obese Her body hair distribution and breast development were normal Her nipples however were pale She had moderate atrophy of the thenar muscles Her lower legs were atrophic and the dorsal extension capacity of the feet was reduced All tendon reflexes were absent She gave an impression of slight mental debility

Oligophrenia and ataxia

Case 3 A man 28 years of age His parents are healthy and there is no consanguinity He is number four of eight brothers and sisters and the only one who is ill He was late in development Thus he was not able to sit until 2 years of age creep at 4 years and walk with support at 15 years When 8 years old he was operated on for bilateral (probably congenital) cataract His speech has always been slurred

Psychometric examinations have yielded values at a low debility level His neurological symptoms have slowly progressed At 27 years of age he was still able to walk but only with support His gait was wide based and unsteady He was very thin and had atrophy and pareses especially in the shoulder girdle around the hips and in the thighs All tendon reflexes were diminished The finger–nose test showed slight ataxia More ataxia was noted at the knee–heel test He had normal body hair but the genital hair had a horizontal upper border The testes were small (2–3 ml)

EMG showed a pattern as in myopathy The motor nerve conduction velocity was normal Pneumoencephalography revealed an extremely small cerebellum with a very large fourth ventricle and cisterna magna and wide pontine angles

Oligophrenia and central motor paresis

Case 4 A man 31 years of age His parents are healthy and there is no known consanguinity The patient has 10 siblings One sister has the same neurological symptoms She could not be evaluated endocrinologically as she has been treated with gestagens during the last few years The patient has a tetraplegia probably dating from birth For a period of two or three years in his childhood he was able to walk with the help of a gocart For the last 10 years he has been confined to a wheel-chair Repeated pshychometric examinations have revealed values at a low debility level

The neurological symptoms of this patient seem to have been stationary for at least the last 10 years with spastic tetraplegia and dysarthria He has flexion contractures in the elbows and knee joints and adduction contractures in the hip joints His body hair is poorly developed and the genital hair has a horizontal upper border The testes are small (1 ml)

EMG including motor conduction velocity of peripheral nerves is normal Pelvic X rays have shown that the pelvic skeleton is very small

Oligophrenia and myopathy

A new syndrome called hereditary myopathy oligophrenia cataract skeletal abnormalities and hypergonadotropic hypogonadism described by Lundberg (20)

Case 5 A man 48 years of age There is no known consanguinity between the parents The patient has eight siblings Two sisters have the same neurological symptoms (cases 6 and 7) The three patients all live at the same institution for the mentally retarded This patient was late in development At 4 years of age he was unable to stand or walk At 9 years he was operated on for bilateral cataract At 16 years he was able to walk His speech was still childish At 36 years of age he had greatly increased difficulty in walking

On examination at 45 years of age he had a large trunk but thin extremities His feet were in a planovalgus position The fourth toes were short The end phalanges of both thumbs were short and broad He had abundant body hair His testes were small (3 ml) his gait was wide based and he had slight intention tremor The shoulder girdle upper arms and lower legs were atrophic and paretic He was not able to get up from the recumbent position without help The tendon reflexes in the legs were normal The biceps and brachioradial reflexes were diminished Babinski's sign was positive

EMG showed a pattern compatible with myopathy The motor nerve conduction velocity was normal At X ray the fourth metatarsal bones were short bilaterally

Case 6 A woman 47 years of age She was late in development At 3 years of age she was not able to walk talk or eat Her tendon reflexes were exaggerated and Babinski's sign was positive At 9 years of age she was operated on for bilateral cataract At that age she was able to walk. She had her menarche at 18 years and amenorrhoea from 21 years of age At 29 years her gait was impaired Her speech was still childish IQ 30 A few years later she was confined to a wheel-chair

On examination at 44 years of age she was small with adiposity of the trunk She had large breasts with pale nipples and normal female body hair She was not able to stand or sit without support and her arms were also weak She had atrophy in the proximal muscles of the arms and legs Her extremities were hypotonic The tendon reflexes in the arms and the patellar reflexes were diminished The achilles reflexes were absent Babinski's sign was positive X ray demonstrated that all metatarsal bones had very small diameters

Case 7 A woman 37 years of age She was late in development At 3 years she was unable to sit stand or talk Babinski s sign was positive At 4 years she was operated on for bilateral cataract At 10 years she was able to sit in a chair but not to stand or walk At 22 years she was thin and had atrophic muscles She has a primary amenorrhoea IQ 30

On examination at 33 years of age she was small with an underdeveloped lower jaw and large mouth She had wide funnel shaped thorax left convex thoracic scoliosis and thoracolumbar lordosis Her pelvis was small She had adiposity of the shoulder girdle The fourth toe of the left foot was short She had normal female body hair and normal breasts but the nipples were pale She was not able to sit without support and could not stand or elevate her arms above the horizontal level

EMG showed myopathic changes A biopsy from the biceps muscle of the arm showed clear changes as in myogenic myopathy X ray disclosed that the fourth metatarsal bone of the left foot was 1 cm shorter than the fifth metatarsal bone of the same foot

Oligophrenia + polyneuropathy + epilepsy (acute intermittent porphyria)

Case 8 A man 39 years of age His mother was mentally retarded The patient himself is mentally retarded at the IQ level of 34 Since a child he has lived in institutions for mentally retarded At 18 years of age he had his first grand mal fit He is rather aggressive especially towards female personnel He masturbates frequently In 1976 his epilepsy got worse with frequent fits his gait was impaired and he had some attacks of abdominal pains

On examination at 39 years of age he was tall with a pectus carinatum His body hair was somewhat sparse with a horizontal pubic line His testes were small (1 ml) His gait was shuffling and his hands and feet were atrophic and paretic The tendon reflexes in the legs were diminished

EEG was pathological with bilateral synchronous epileptogenic episodes EMG in hands and feet showed a pattern compatible with a peripheral neurogenic lesion The motor nerve conduction velocity in the left peroneal nerve was 39 m/sec Chemical analyses of the blood and urine have indicated that the patient suffers from acute intermittent porphyria He has an elevated urinary excretion of ALA and PBG The activity of uroporphyrin-1 synthetase is 50% of normal

RESULTS OF HORMONE ASSAYS

The results are given in Table II The gonadotropic prolactin and testosterone levels in serum were assayed in seven of the eight subjects FSH was raised in them all and LH in all but one who had a normal level in serum LH RH tests were performed in six cases and significant LH responses were seen in them all while FSH was further increased in three out of five cases Two of the four men had a low testosterone level in serum and two had a level within the normal range The prolactin level was normal in all subjects

DISCUSSION

The endocrine symptoms were very similar in all patients The four males had normally developed external genitalia but pronounced testicular atrophy (testicular volume 1–3 ml each) The testes had

Table II *Results of endocrine investigations in eight cases of hypergonadotropic hypogonadism oligophrenia and five different neurological syndromes*

Sella turcica adrenocortical and thyroid function normal in all
LH=luteinizing hormone FSH=follicle stimulating hormone LH RH=LH releasing hormone

Pat no and karyotype		Sex and age (y)	Main neurological symptoms	Endocrine symptoms	LH RH test				
					LH (μg/l)		FSH (μg/l)		
					Basal	Response	Basal	Response	Prolactin (μg/l)
1 46 XX	Siblings	♀ 63	Oligophrenia polyneuropathy	Menarche at 12 y Menopause at 25 y	5 25	+10 25	10	–	5 5
2 –	Siblings	♀ 57	Oligophrenia polyneuropathy	Menarche at 13 y Menopause at 18–20 y	–	–	–	–	–
3 46 XY		♂ 28	Oligophrenia ataxia	Atrophy of the testes (2 resp 3 ml)	4 5	+17 0	11	+4 5	3 9
4 46 XY		♂ 31	Oligophrenia spastic tetra plegia	Atrophy of the testes (1 ml)	6 75	+ 8 75	14	+1	5 9
5 46 XY	Siblings	♂ 48	Oligophrenia mus cular dystrophy	Atrophy of the testes (3 ml)	3 5	+ 5 15	5 8	+1 95	9 6
6 46 XX	Siblings	♀ 47	Oligophrenia mus cular dystrophy	Menarche at 18 y Menopause at 21 y	10	–	17	–	5 7
7 46 XX	Siblings	♀ 37	Oligophrenia mus cular dystrophy	Primary amenor rhoea	7 2	+10 8	13 0	+2 5	6 3
46 XY		♂ 39	Oligophrenia epi lepsy poly neuropathy	Atrophy of the testes (1 ml)	2 5	+ 2 35	6 3	+0 8	1 4

probably never been normal On the other hand the testosterone concentrations in the blood though decreased were not very low (4 2–15 6 nmol/l) and body hair was present to an almost normal extent Three of the females had their menarche at a normal age but a very early menopause (18–25 years) while the fourth had a primary amenorrhoea However the breasts and body hair were normally developed LH and FSH values were very high as in Klinefelter s and Turner's syndrome respectively but the karyotypes were normal The response to LH RH was enhanced as in Klinefelter patients (22) or in the normal menopause (21) There were no signs of thyroid or adrenal insufficiency and the prolactin values were normal Thus the endocrine evaluation revealed no signs of hypothalamo-pituitary insufficiency Instead the endocrine insufficiency was confined to the gonadal system and was of a similar type as that in gonadal dysgenesia or premature gonadal ageing

Hypogonadism has been described in a number of hereditary neurological syndromes (44) in combination with cerebellar ataxia (1 9 12 23 26 41 45) neural progressive muscular atrophy (13) oligophrenia and Charcot Marie Tooth syndrome (14) Charcot Marie Tooth syndrome (39 42) ataxia

-sto-erone mol/l)	Oestradiol (pmol/l)	Height (m)	Weight (kg)
38	40	1 36	56 9
	–	1 36	–
l 6	100	1 68	49 0
l 2	45	1 54	32 5
5 6	–	1 64	63 8
4 5	–	1 50	71 6
l 08	Oestrogen (urine) 6 8 µg/ 24 h	1 70	49 8
5 71	187	1 70	70 0

teleangiectasia (2 25) Laurence Moon Bardet Biedl syndrome (3 15 30) Prader Labhart Willi syndrome (11 24 43 49) idiotia xerodermica (34) Marinesco-Sjogren syndrome (38) Moebius syndrome (27) myotonic dystrophy (4 5 8 33 35) muscle dystrophias of pelvic girdle types (10) and ocular myopathy (17 18) as well as in the two syndromes with oligophrenia and polyneuropathy or oligophrenia and myopathy respectively described by Lundberg (19 20)

In many of these cases available endocrinological data are few and the evaluation is therefore difficult Most cases seem to have had a hypogonadotropic hypogonadism However in most cases with myotonic dystrophy in some cases with ocular myopathy in one type of Laurence Moon Bardet Biedl syndrome in the Marinesco-Sjögren cases described by Skre et al (38) as well as in the present eight cases the hypogonadism was clearly hypergonadotropic Thus in at least nine different neurological syndromes all probably hereditary hypergonadotropic hypogonadism may be present

The neurological symptoms are different in these syndromes the endocrine symptoms however appear to be similar One likely explanation is that the patients may have a hypogonadism due to a primary genetic defect of the gonads However the possibility of a hypogonadism secondary to a hypergonadotropism should also be considered Testicular damage has been observed after administration of gonadotropins to humans (28) and rats (40) Two of the patients in the present paper (cases 3 and 5) with high levels of both FSH and LH had normal levels of testosterone in blood This is a very rare picture which is seen in patients with the syndrome of complete testicular feminization It is thought in these cases that the hypothalamic control centres as well as the peripheral target organs are insensitive to testosterone There is evidence that gonadotropins may be involved in the regulation of their own receptors in the gonads (29 37) A loss of receptors and decreased sensitivity of the gonads may be the result of long excessive exposure to gonadotropins One may speculate that some of the patients in the present study have a hypergonadotropism due to an insensitivity of the hypothalamus (or pituitary) to the negative feedback action of the gonads and then a hypogonadism as a result of an overstimulation by gonadotropins

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Prophylactic Treatment with Miconazole in Patients Highly Predisposed to Fungal Infection

A Placebo-Controlled Double Blind Study

Hans Brincker

From the Department of Oncology and Radiotherapy Odense Hospital Odense Denmark

ABSTRACT In a placebo controlled double blind study the prophylactic value of oral systemic treatment with the antimycotic agent miconazole was assessed in 30 highly predisposed patients receiving intensive cytostatic chemotherapy because of haematological malignancies Patients colonized with Candida before treatment were not freed from this micro-organism by miconazole treatment However, only 3 out of 6 initially non-colonized miconazole treated patients became colonized during the study, against 10 out of 10 placebo-treated patients ($p=0\,036$) Seven out of 15 patients in the placebo group developed clinical mycosis, against only two out of 15 in the miconazole group The miconazole treated patients remained clinically free of mycosis for 252 out of 264 treatment days, while the placebo-treated patients remained free of mycosis for only 263 out of 338 treatment days ($p=0\,0001$) The results indicate that systemic miconazole treatment protects highly predisposed patients from colonization with Candida and prevents or postpones clinically established candidosis

Intensive cytostatic therapy in patients with haematological malignancies usually induces both marked granulocytopenia and immunosuppression These two factors probably constitute the main reasons for the high incidence of fungal infections in patients submitted to this type of therapy

Miconazole a broad spectrum antifungal agent (2) belonging to the class of imidazole antimycotics is widely used in the topical treatment of mycoses Moreover its systemic use has been reported by several investigators (3 4 5 6 7 8 9 12 13 15 17 18 20 21 23 26) Of special relevance to the study reported here are successes obtained with this drug in the treatment of mycoses of the digestive tract (22 24 25) its successful use as an oral treatment of South American blastomycosis (14) and uncontrolled observations (1 11) suggesting the usefulness of miconazole in highly predisposed patients with haematological malignancies

The aim of this study was to investigate the latter possibilities by means of a placebo-controlled double blind evaluation of miconazole s ability to protect highly predisposed patients against fungal infections

STUDY POPULATION AND METHODS

Patients (Table I)

Thirty patients with leukaemia or leukaemic lymphoma were included in the study that took place between Aug 1975 and Feb 1977 All were highly predisposed to fungal infections as a consequence of intensive cytostatic treatment resulting in granulocyte counts below 1000/μl for several days

Only patients presenting no clinical signs of fungal infection were eligible for the study but a previous oral candidosis did not rule out participation provided that this infection had been successfully treated before the patient entered the trial

Treatment

Eligible patients were randomized to receive either a placebo or miconazole 500 mg four times daily The drug was given as tablets of 250 mg which were separately packed for each patient in sufficiently large amounts to ensure double blindness The tablets were to be swallowed and the study protocol prescribed this treatment to be continued for at least three weeks (maximum 25 days)

Patients who developed signs of localized candidosis of the oral and/or oesophageal mucosa received local treatment with amphotericin B while the treatment with the placebo or miconazole was continued However in patients developing signs of generalized mycosis the randomization code was broken for the patient concerned

Table I *Results of randomization*

AML=acute myeloid leukaemia ALL=acute lymphocytic leukaemia

	Miconazole group	Placebo group	p value
Sex (♂/♀)	8/7	8/7	n s *
Age (below/over 54 y)ᵃ	8/7	7/8	n s **
Diagnosis (*myeloid/lymphoid*)	12/3	10/5	n s *
Myeloid AML primary	6 } 12	8 } 10	
AML relapse	6	2	
Lymphoid ALL primary	2 } 3	3 } 5	
ALL relapse	0	2	
Leukaemic lymphoma	1	0	
Very poor general condition	5 of 15	2 of 15	n s *
Prior buccal mycosis	9 of 15	3 of 15	0 06*
Incidence of colonization			
Oral	4 of 15	4 of 15	n s *
Anal	5 of 15	2 of 15	n s *
Concurrent antibiotics	14 of 15	13 of 15	n s *
Lowest granulocyte count (below/above 48/μl)ᵃ	6/9	9/6	n s *

ᵃ Median of total population

* Fisher exact probability test. n s = $p>0$ 10

** Median test n s = $p>0$ 10

and she or he was then given systemic therapy with amphotericin B 5-fluoro-cytosine and/or miconazole

Concurrent antimicrobial treatment was given in 27 of the patients (Table I) because of suspected or verified bacterial infection This treatment consisted of a triple combination of tobramycin penicillin V and sulfamethoxazole/trimethoprim

Prednisolone was given in all eight patients with lymphoproliferative malignancies but in none of the 22 acute myeloid leukaemia (AML) patients

possible oral as well as systemic infections was carefully registered during the treatment for all participating patients

Also cultures were taken from the oral cavity and the anal canal (faeces) of all patients at the start of the study and weekly thereafter The growth on the culture media was assessed as 0=no growth F=a few colonies (not found in this study) S=several colonies M=massive growth A patient was considered to be colonized by fungi as soon as growth was observed on at least one culture and the highest degree of fungal growth on the cultures was used as a measure of the severity of this colonization In patients developing signs of fungal infection cultures were also taken from the sputum urine and blood A clinical evaluation was performed in all patients every day a leukocyte and differential count at least twice weekly a chest X-ray was taken once weekly and serum IgA IgG and IgM were determined at the start of the study and after three weeks

Statistical evaluations

The Fisher exact probability test and the median test were used for assessing the results of the randomization procedure

The colonization rate during the treatment was evaluated using the cultures which had shown the most pronounced growth Since it was found that colonization with Candida species took place after a mean interval of about 1 1/2 weeks five patients (four from the miconazole and one from the placebo group) who had been treated for less than a fortnight were not considered for the analysis of this aspect Therefore at least two double (i e oral and anal) cultures were available from each patient during double-blind treatment. The Fisher exact probability test was used to compare the two treatment groups regarding 1) the proportion of patients who became colonized during the treatment and who had shown negative cultures at the start 2) the greatest cumulative difference between the two groups after subdividing the populations into patients who became positive who remained either positive or negative and who had yeasts before the study but who no longer showed any growth on the cultures during the study

As the duration of treatment was not entirely similar (though not reaching the 5% level of statistical significance) between the two treatment groups it was considered unfair to make a straight-forward comparison of the total number of patients who became infected in the two groups Two approaches were used to correct this difference in the duration of treatment (which was also the duration of observation) 1) The total number of protected days was calculated for each treatment group and related to the total duration of the observation The intergroup difference was assessed by means of the χ^2 test 2) The total duration of protection for each patient was also used for an overall assessment of the infection rate in each treatment group by means of the actuarial analysis technique These results were statistically evaluated by means of Student s t test Two-tailed probabilities were calculated for all statistical tests used

Table II *Reasons for stopping treatment before 3 weeks*

ALL=acute lymphocytic leukaemia AML=acute myeloid leukaemia LL=leukaemic lymphoma

Treatment	Trial no	Sex	Age (y)	Basic disease	Treatment stopped on day	Reason
Placebo	4	♂	67	ALL	8	Systemic candidosis
	23	♀	71	AML	17	Herpetic stomatitis
	24	♂	77	ALL	14	Death
Miconazole	3	♀	57	AML	20	Systemic aspergillosis
	5	♂	49	AML	18	Poor condition
	20	♀	71	AML	14	Poor condition
	7	♂	65	AML	12	Poor condition
	22	♂	65	AML	9	Death
	9	♂	19	ALL	4	Poor condition
	29	♀	57	ALL	18	Discharged
	18	♀	43	LL	16	Discharged
	21	♂	21	AML	5	Error

RESULTS

Results of the randomization procedure (Table I)

Though the two treatment groups proved to be comparable as they did not differ significantly for any characteristic the active drug was disadvantaged by a somewhat uneven distribution more patients in the miconazole group had had oral candidosis before and somewhat more patients in the same group were in a very poor condition probably because of a higher number of relapse cases of AML This is also reflected in the fact that nine patients in the miconazole group had their treatment interrupted within three weeks against only three in the placebo group (Table II) Moreover the anal pretreatment colonization rate tended to be somewhat higher in the miconazole than in the placebo group On the other hand the above findings were partly counterbalanced by a somewhat lower granulocyte level in the placebo patients

Fungal colonization rate

As explained above 25 patients (11 treated with miconazole and 14 controls) were considered evaluable as they had been treated for at least two weeks The degree of colonization by Candida spp before and during therapy is shown in Fig 1 Although the pretreatment degree of colonization is somewhat to the disadvantage of the miconazole patients especially regarding the coprocultures this pattern is reversed during treatment When growth on culture is used as a yes or no phenomenon it is found that only three out of six initially negative (combined data) miconazole patients became positive during the study against all ten placebo patients ($p=0\ 036$) Also if the patients are ranked according to the changes observed in their cultures during the double blind treatment (Table III) significantly more control patients are colonized at the end of the study ($p=0\ 047$) Similar trends are seen when the oral and anal cultures are considered separately but these differences fail to reach the level of statistical significance though the difference regarding the coprocultures was nearly significant ($p=0\ 090$)

Occurrence of mycotic infections

Two patients in the miconazole group developed a mycosis after 15 (oral candidosis) and 20 days (generalized aspergillosis) respectively Seven placebo patients experienced fungal infections six of whom developed oral candidosis after 3–19 days (median 14) of treatment followed by a generalized candidosis eight days later in one the seventh patient developed a generalized aspergillosis on day 19 All the cases of oral candidosis responded well to topical application of amphotericin B but all three generalized mycoses were fatal despite systemic antifungal treatment Neither chest X rays nor cultures from sputum and urine were helpful in establishing the diagnosis of generalized mycosis in these patients and positive blood cultures were

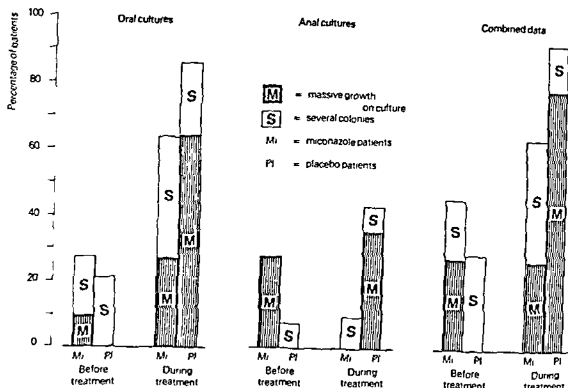


Fig 1 Presence of Candida species in cultures

obtained only in a 67 year-old male acute lymphocytic leukaemia patient who died of generalized candidosis Also possible abnormalities of the immunoglobulin levels did not correlate with the occurrence of the fungal infections (nor with the ionization rates)

Miconazole treated patients had been followed during treatment for 264 days and had remained clinically free of mycosis for 252 days In contrast the placebo-treated patients who had been followed for 338 days had remained free of such diseases for only 263 days This difference is highly significant ($p<0\ 0001$) Further according to the results of the actuarial analysis (Fig 2) the percentage of patients who had developed a clinical mycosis was significantly higher in the control group than in the miconazole group after 14 and 19 days of treatment ($p<0\ 05$) The latter analysis also shows that one quarter of the control patients had already developed a mycosis after 13–14 days whereas this occurred about one week later in the miconazole group However the duration of the treatment was too short to determine whether miconazole therapy protects a certain number of patients from mycotic infections or only postpones the occurrence of such diseases

In order to rule out the possibility that the observed difference was due to a lower granulocyte count in the control group the data were analyzed separately for the six miconazole patients and the

Table III *Colonization rate changes observed during double blind treatment*
Patients are considered positive as soon as fungal growth has been found on one of the cultures (either oral or anal)

Group	Positivation	No change		Negativation	Total no of evaluable pats
	– before → + during	+before → + during	– before → – during	+ before → – during	
Miconazole	3	4	3	1	11
Placebo	10	3	0	1	14

• The greatest cumulative difference is seen here $p=0\ 047$ (Fisher exact probability test)

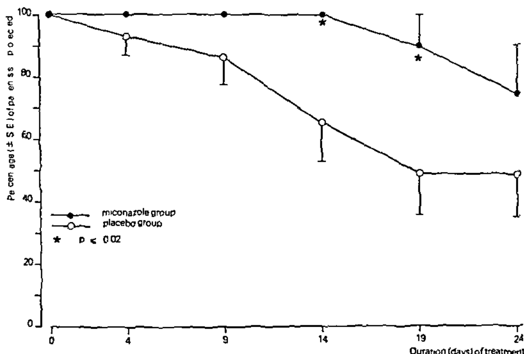


Fig 2 Duration of protection against fungal infections

nine control patients whose granulocyte counts were below 48/μl All the patients who had developed a clinical mycosis during the study belonged to this category *with the exception of one* patient from the placebo group with an oral candidosis The six miconazole patients whose granulocyte counts were below 48/μl had been protected *for 84 days during their treatment* which had lasted for a total of 96 days whereas the corresponding nine placebo patients had remained free of clinical mycosis for only 144 of their 207 treatment days The difference between these two groups is statistically significant ($p = 0\ 001$)

Side-effects ascribable to the miconazole treatment were not seen either in this or in a previous study (1)

DISCUSSION

The results of this double blind study indicate that oral systemic miconazole treatment protects highly predisposed patients from colonization with Cand da spp Further such patients are protected by this treatment against clinical infections with Candida or the occurrence of such infections is postponed These data were obtained even though the randomization procedure had sl ghtly disadvantaged the active drug except for the absolute granulocyte count which tended to be lower in the *control group However it was found that the latter* factor could not explain the overall results

In the dose employed the effect of the miconazole treatment appears to be prophylactic *rather than curative since it was found that patients* who were colonized with Candida at the beginning of the treatment were not freed from this micro-organism more often than similar patients treated with a placebo (Table III) Whether the protective effect of miconazole is directed against types of fungus other than Candida remains to be examined

The importance of the protective effect of miconazole demonstrated in this study is quite obvious since a period of prolonged granulocytopenia usually is inevitable during chemotherapeutic induction of remission in acute leukaemias Further the ease of administration and the low rate of side effects of miconazole compare very favourably with other antimycotics intended for systemic use

As the effect of the treatment was more marked on the anal than on the oral cultures and as the tablets used in this study were swallowed by the

patients it may be speculated that still more efficient prophylaxis could be achieved if this type of treatment were supplemented with a concomitant topical treatment of the oral mucosa In one open study systemic treatment with miconazole tablets together with topical oral application of amphotericin B was used and the results suggested an additive effect (11) Also since the digestive tract is generally considered to be an important site of entry for systemic candidosis in highly predisposed patients (10, 16 19) one could expect that a longer lasting treatment in patients who remain highly predisposed for a long period of time may significantly prevent the occurrence of systemic candidosis Finally it must be borne in mind that the systemic use of prophylactic miconazole treatment may have ecologic consequences which cannot be assessed at present

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LETTER TO THE EDITOR

TURNOVER STUDIES WITH RADIOIODINATED ALBUMIN IN UREMIC PATIENTS

Sir

A recent paper published by S V Johansson et al in Acta Medica Scandinavica (7) deals with the determination of serum albumin metabolism in 10 patients with chronic uremia using a radioiodinated tracer The results show an increased ratio of the extravascular to the intravascular albumin pools (EV/IV)—2 8 compared to 1 16 in normal controls—and therefore an increase of both the extravascular and the total pools of albumin in addition a reduced catabolic rate of the protein is described in the uremics compared to the normal value (7 4 and 11 4 g/day respectively)

These observations are in marked contrast with our earlier turnover studies performed in a large series of uremic patients in whom we found a significantly reduced EV/IV ratio (0 86 compared to the normal value of 1 5) together with catabolism values that were decreased in proportion to the reduced total albumin pool values (1 3) Johansson et al try to explain these discrepancies on the basis of some pathophysiologic mechanism (more or less severe dietary protein intake) while they almost ignore methodological differences as the possible reason Instead we believe that the observed discrepancies depend entirely upon important methodological differences in the data analysis particularly with respect to iodide kinetics in renal failure In fact Johansson et al calculate fractional albumin catabolism as the ratio between the inorganic radioactivity excreted into the urine in 24 hours and the mean plasma activity of labelled albumin during the same period (U/P ratio method (9)) However such a method assumes that radioactive iodide released from the tracer degradation is excreted promptly into the urine as soon as catabolism of the protein takes place and it can be accepted as valid only in subjects with normal renal function Indeed iodide excretion by the kidney is greatly and progressively impaired in patients with renal failure so that radioiodide is retained in the body to a large extent In these conditions the magnitude of the numerator of the catabolism formula U is greatly reduced and the procedure grossly underestimates the fractional catabolic rate of albumin Iodide retention due to renal failure also greatly affects the determination of the EV/IV ratio by the retained dose method (5) employed in the paper cited (7) In fact free radioiodide released from albumin catabolism and retained in the body of patients with renal failure is treated erroneously as intact tracer protein in the measurement of the retained dose This obviously leads to a large overestimation of the extravascular activity as computed by the retained dose procedure and consequently the EV/IV ratio is likewise largely overestimated

All these shortcomings in the use of radioiodinated tracers for metabolic studies in patients with renal failure are overcome by applying the two-tracer method which is based on the injection of a tracer for the free iodide system together with alternatively radioiodinated albumin (2 4 6) The free iodide tracer enables one to calculate the kinetics of iodide in the individual patients in relation to their degree of residual renal function and therefore in each study to correct the behaviour in plasma and urine of iodide released from albumin catabolism In this way the delayed iodide excretion in renal failure is fully taken into account in the computation of the turnover and distribution parameters of the labelled protein

In order to test the hypothesis that a different mathematical treatment of the experimental data was solely responsible for the metabolic differences observed between the uremic patients studied by Johansson et al and our patients we re-examined 20 of our 62 uremics previously submitted to albumin turnover investigation by the two-tracer method (3) The experimental data for these 20 patients were analysed in the manner used by Johansson et al and the catabolism and distribution constants were compared with those obtained by the two-tracer method

The results obtained with the two computational procedures (listed in detail in Table I) showed a clear systematic underestimation of catabolism by the U/P technique in relation to the two-tracer method with a mean value of 53 4%±23 2 S D ranging from a minimum of 1 3% to a maximum of 90 2% As expected on the basis of the above considerations the EV/IV ratio values computed by the simple retained dose procedure were gross overestimates compared with the two-tracer method (mean overestimation 145 3%±113 5 S D from 3 4% to 371%)

Thus it seems obvious that in uremic patients the use of relatively simple computational procedures like those described by Johansson et al leads to gross miscalculations of both catabolism and distribution of radioiodinated albumin because of the iodide retention produced by renal failure which affects these metabolic parameters in two opposite ways namely by underestimating catabolism and overestimating the extravascular pool of the protein Furthermore it is not possible to correct the experimental data obtained in these conditions by applying an average estimate of the kinetics of free iodide in renal failure as mentioned by Johansson et al because iodide kinetics vary greatly and unpredictably in the individual uremic patients and iodide retention itself is not strictly dependent on the degree of impairment of renal function (8) Thus the simultaneous injection of a tracer of the free iodide system as in the two-tracer method remains essential for a correct definition of the iodide kinetics in the individual patients

Table I Comparison of the catabolism and distribution results obtained in the same uremic patients by the U/P ratio and retained dose technique vs the two tracer method

Case no	Catabolic rate (mg/kg/d)		Underestimation by U/P technique (%)	EV/IV by retained dose technique	EV/IV by two-tracer method	Overestimation by retained dose technique (%)
	By U/P ratio	By two-tracer method				
030	55	135	59 3	1 40	0 91	53 9
032	156	222	29 7	1 75	1 21	44 6
034	41	185	77 8	1 52	0 74	105 4
042	55	160	65 6	1 69	0 74	128 4
195	66	134	50 7	1 70	0 55	209 1
209	125	167	25 1	2 02	1 27	59 0
214	64	145	55 9	1 72	1 13	52 2
216	51	198	74 2	2 06	0 95	313 8
219	23	103	77 7	1 70	1 24	371 0
221	102	117	13	3 00	1 20	150 0
223	130	180	27 8	1 41	0 82	71 9
225	153	255	40 0	1 54	0 96	60 4
228	61	226	73 0	1 71	1 15	48 7
236	284	354	19 8	1 18	0 36	227 8
237	65	178	63 5	1 74	0 86	102 3
247	15	153	90 2	2 00	0 47	325 5
248	93	267	65 2	1 87	0 53	252 8
249	42	78	46 1	1 38	0 97	42 3
265	62	189	67 2	1 00	0 26	284 6
271	29	84	65 5	1 51	1 46	3 4
Mean	83 6	176 5	53 4	1 695	0 89	145 3
±S D	62 5	66 4	23 2	0 41	0 33	113 5

so that the various turnover and distribution parameters of albumin can be determined accurately from plasma urine data particularly if the experimental interval to 6–8 days with the aim of approximating ly state conditions throughout the study

Mariani F Bonaguidi and R Bianchi
2nd Medical Clinic C N R Clinical Physiology Laboratory and Institute of Metabolic Diseases of the University of Pisa Pisa Italy

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Idiopathic Acquired Panmyelopathy in Pregnancy

A Case Report

J P Samsom Th M Brouwers H J Huisjes G S van der Schans and H O Nieweg

From the Bonifatius Hospitaal Leeuwarden and the Division of Haematology Department of Internal Medicine and Obstetrical Department University Hospital Groningen The Netherlands

ABSTRACT A case of idiopathic acquired panmyelopathy (aplastic anaemia) in pregnancy in a 32-year-old woman is presented The outcome for mother and child was favourable Special attention is paid to the management of the delivery Another aspect of interest is the haematologic status of the child In panmyelopathy an immunologic mechanism may operate as indicated by the finding of platelet membrane associated immunoglobulins, which were also present in our patient If the bone marrow lesions in this disease are indeed due to immunological injury, they are mediated by immune complexes or by a cellular mechanism for it is well known that IgG antibodies may pass through the placenta and induce lysis of circulating blood cells in the newborn There was no evidence of bone marrow damage in the infant This is in accordance with the cases mentioned in the literature

Though the first case of panmyelopathy (aplastic anaemia) described (7) was that of a pregnant young woman the association of idiopathic panmyelopathy—an uncommon disease anyway—and pregnancy is rare From a review of the published cases (8) it appears that the outcome for mother and child is often fatal The author states that 28 out of 41 mothers died and that not more than 20 infants survived

The management of the patient with severe panmyelopathy is known to be exacting When pregnancy and delivery are complicating factors the problem is even more difficult The availability of effective bactericidal drugs as well as the development of modern blood banking techniques and the possibility of selecting compatible donors for platelet transfusions have however lessened the dangers

In the present paper we present an additional patient with panmyelopathy and pregnancy and report the method chosen for managing the delivery The child who had an inspissated bile syndrome with functional obstruction of the large intestine did not show bone marrow damage The implications of the haematologic findings in this child and the other children reported in the literature (Table I) are discussed in the light of a possible immunologic mechanism for the bone marrow damage in idiopathic panmyelopathy

CASE REPORT

In Sept 1971 a 32 year-old woman gravida 4 para 2 was admitted to the hospital in the 27th week of her pregnancy because of anaemia leukopenia and thrombocytopenia She had been healthy till a year before At that time she observed haematomas after slight trauma There was no exposure to irradiation or chemicals Drugs had been taken from a few months before admission acetylsalicylic acid phenacetin and coffein on her own initiative as well as folic acid vitamin B_{12} and iron on medical prescription A dental extraction seven weeks before admission resulted in profuse haemorrhage She was treated by transfusion of seven pints of whole blood in another hospital

On admission in the Haematology Ward she was not acutely ill There was a deep pallor No enlarged lymph nodes could be palpated Liver and spleen were not enlarged Multiple bruises and purpuric spots were seen on the lower extremities In the fundus of the eyes there were no haemorrhages The height of the fundus uteri was consistent with 27 weeks of gestation Apart from the abnormalities in the blood picture and in the bone marrow biopsy laboratory data were within the normal range (Table II)

A diagnosis of idiopathic panmyelopathy was made In cooperation with the staff of the Department of Obstetrics and Gynaecology a plan was made for the further management

From the 27th till the 36th week of her gestation no

Table I *Haematological data of live born children to mothers with panmyelopathy compiled from the literature*

	Hb (g/100 ml)	Htc (%)	Erythrocytes (×10⁶)	Reticulocytes (‰)	Leukocytes	Thrombocytes
Bigby and Jones (2)	13 4 (4th day)	–	6 68	–	–	–
Born (3)	–	–	2 4	–	14 000	–
Dorgan and McGaughey (6)	"Blood studies normal"					
Loring (18)	"Blood studies essentially within normal limits"					
Rovinsky (22)	19.5	60	6 05	55	10 900	112 000
Kyser and Danforth (15)	19 4	56	–	–	–	–
After 4 days	17 7	50				
Klonowicz et al (12)	–	–	–	–	18 000	143 000
Rosner and Sussman (21)	17 7	58.5	–	3	10 500	200 000
Taylor et al (26)	17 2	–	–	–	after 4 days	167 000
Collins et al (4)	–	62	–	–	14 000	44 000
				Iso-antibodies	4 days	12 000
					20 days	40 000

major complication occurred Leukocyte poor packed cells were given to keep the Hb level above 6 g/100 ml and at one time a platelet transfusion was administered because of a nasal bleeding The platelet count did not rise sufficiently in relation to the amount given Tests for leukocyte antibodies at this time were positive against 5 of 20 random leukocyte suspensions and negative against a suspension of her husband Her family was typed for HL-A antigen The father differed from the patient in only one antigen and one brother turned out to be HL-A identical

In the 36th week the patient was delivered by caesarean section of a living male child After this a hysterectomy was performed The operation was carried out after ad tration of 6 U of platelet concentrate harvested by et pheresis from the father and the HL A identical 8 U platelet concentrate obtained in the usual from individual donors selected by a negative cross-between their lymphocytes and serum of the pa tient About 4 hours postoperatively she received again 8 U of platelet concentrate from selected individual donors Because the platelet donors including her father and brother were all Rh positive the patient who was Rh-negative received a small number of Rh-positive red cells For suppression of Rh immunization anti Rh immunoglobulin was administered

During the whole operative procedure there was minimal bleeding Recovery was complicated by a period of fever ascribed to infection which however could not be localized and for which she received antibiotics On three occasions in the first postoperative week she re ceived platelet concentrates from her father and brother to prevent haemorrhage from the site of operation

The blood picture did not improve after delivery One year later a laparotomy had to be performed because of a mass in the lower abdomen This turned out to con sist of multiple pseudo-cysts filled with old blood and adherent to several loops of the intestine and the ovaries

The child had a weight of 2440 g and an Apgar score 9 at 1 min and 10 at 3 min His blood group was AB negative It showed a nearly normal blood picture (Table III) and had signs of mild hyaline membrane disease After three days laparotomy had to be performed because of signs of bowel obstruction. At operation the distal colon appeared not to be functioning and a colostomy was made Biopsies from the colon were normal A liver biopsy taken at the age of 3 months because of jaundice showed the picture of an inspissated bile syndrome This turned out to be transitory and after restoration of the continuity in the lower bowel his further development was normal

METHODS

Routine haematological data were obtained using standard techniques HL-A typing and determination of leukocyte iso-antibodies were carried out in the Special Laboratory for Blood Group Serology of the Groningen University Hospital by means of an agglutination technique and a microcytotoxicity technique (11) The serum was tested for platelet antibody by the Pf3 method as described elsewhere (24) The platelets of the patient herself were tested for platelet membrane associated IgG by a direct immunofluorescence technique Platelets were separated from plasma by gel filtration on Sepharose 2[B] (25) Immunoglobulins associated with the platelet membrane were detected semiquantitatively by staining with fluorescein-conjugated monospecific anti-human globulin sera

DISCUSSION

In the literature 41 patients with idiopathic (?) panmyelopathy in pregnancy have been described Reviews have been given in several publications (8 16, 19 22 26)

The first aspect of the association of panmyelo-

Table II *Laboratory values on admission*

Blood group	A Rh neg
Hb	8 0 g/100 ml
Leukocytes	2 400/mm³
Differential count	31% PMN granulocytes 64% lymphocytes 5% monocytes
Platelets	5 000/mm³
Reticulocytes	8‰
Erythrocytes	2 5 mill /mm³
Sternal aspiration	Moderate erythropoiesis without megaloblastic features very few megakaryocytes normally active myelopoiesis
Iliac crest biopsy	Light microscopy Fatty marrow with very little erythropoietic or myelopoietic activity no signs of myelofibrosis or tumour Electron microscopy Normal bone marrow sinusoidal walls
Direct Coombs test	Negative
Ham test	Negative
Foetal Hb	Not present
Urobilinogen excretion in faeces	Normal
Renal and liver functions	Normal
X ray thorax	No thymoma

pathy and pregnancy which should be discussed is the management of the pregnant patient with this disorder Though the treatment has to be symptomatic a number of measures may contribute to a good result Agents known to be capable of inducing bone marrow damage should be avoided The blood Hb is to be kept at a level at which the patient is free of anoxic symptoms using judicious administration of red cell suspensions from which leukocytes and platelets have been removed as far as possible This is done in order to prevent the development of leukocyte and platelet iso antibodies Technical details are given in recent reviews (5)

Another effect of bone marrow damage is the danger of infection due to granulocytopenia Some simple measures can be taken to decrease the chances of infection e g disinfection of the skin and the nares More aggressive methods such as eradication of the intestinal microflora are probably better avoided as long as there is a significant number of granulocytes (>500/mm³) One should however always look for latent or incipient infections

Especially during the period of pregnancy platelet concentrates should be used sparingly and only when there is a definite need due to a bleeding tendency Platelets from random donors are liable to stimulate the development of iso-antibodies If possible platelets from donors sharing HL A antigens with the father should not be used In the present state of our knowledge we have to consider the possibility that the use of platelets from HL-A typed donors usually siblings of the patient does not prevent the induction of antibodies against antigens specific for platelets only

Antibodies with affinity for platelets are dangerous because they inactivate transfused platelets when they are needed for haemostasis at the time of the delivery and also because they may induce thrombocytopenia in the child In pregnant patients who have had the chance of iso-immunization it may prove to be of interest to test the maternal serum for the presence of antibodies with affinity for the father's platelets A very sensitive technique for detecting platelet iso-antibodies was developed recently (23) Perhaps such a test may give some information about the chance that transferred maternal iso-antibodies affect the child s platelets

In the non pregnant individual with panmyelopathy anabolic steroids have been used extensively even though the evidence for their effectiveness in acquired panmyelopathy is contradictory Moreover their administration for some months at least in the amounts generally used in panmyelopathy is a risky procedure in pregnancy

The modern therapy of bone marrow transplantation can only be used when the patient is first seen in a stage when therapeutic abortion is still feasible The immunosuppressive drugs necessary before and after the administration of compatible bone marrow cells would endanger the child The management of the pregnant patient with panmyelopathy is therefore in our opinion essentially conservative Its purpose is to reach a stage in pregnancy when delivery can be induced with safety for the child

Table III *Haemogram of the child*

	1 hour p p	24 hours p p	1 week p p
Htc (vol %)	28	59	60
WBC/mm³	12 600	13 000	15 000
Platelets/mm³	119 000	120 000	200 000

The second point which should be discussed is the management of the delivery itself As far as possible this has to provide a maximal degree of protection to mother and child during this critical period against the dangers confronting them

The main threat to the woman is bleeding either uncontrolled external blood loss or intracranial haemorrhage Maximum protection can only be provided when large amounts of platelets liable to be effective are available therefore delivery should occur at a predetermined time Premature labour can be postponed by betamimetic drugs if necessary in combination with ethanol infusion Although not successful in all cases in the majority a sufficient delay can be achieved for preparation of platelet concentrates In the case of our patient it was decided to perform caesarean section We believed it to be advantageous to avoid straining with the associated increased pressure in the intracranial vasculature Another reason for choosing this method of delivering the child was that external pressure on the head of the child and the attendant cranial deformation were avoided as far as possible In the present case the child was born with an adequate number of platelets but transfusion induced platelet iso-antibodies in the mother might have provoked thrombocytopenia in the foetus which would make it highly vulnerable to intracranial haemorrhage

Hysterectomy was done because the patient and husband did not want more children and stop menstrual blood loss would lessen transfusion 'irements A potential disadvantage was a risk internal blood loss with an intra abdominal wound

The next question concerns the significance of the observations of pregnant women and their offspring for our understanding of panmyelopathy Concerning the possible etiology it is usually assumed that there is no causal relationship between the two conditions In a critical review (19) it is stated that in most reports in which an association between pregnancy and panmyelopathy was claimed the arguments and data are not convincing This also applies to the case report (19) in which a bone marrow biopsy between the pregnancies was not reported and therefore a mild form of panmyelopathy aggravating under the stress of gestation cannot be excluded In our patient the blood disorder persisted after delivery making it unlikely that pregnancy was the causative factor

An immunological mechanism has been postulated for panmyelopathy (13) This does not seem improbable in view of its association with thymoma and systemic lupus erythematosus both conditions with autoimmune features The role of antibodies in the pathogenesis of pure red cell aplasia has already convincingly been demonstrated (10 14) In panmyelopathy—both of the idiopathic and of the drug induced type—the occurrence of antibodies which affect the platelets in the presence of a sulphydryl inhibitor, parachloromercuribenzene sulphonate (PCMBS) was described (20) The serum of the mother was positive when tested in this system More recently a simple technique was developed for detecting platelet membrane associated immunoglobulins Platelets are exposed to fluorescein conjugated antibodies to human immunoglobulins after gel filtration for the removal of plasma proteins With this method immunoglobulins were found at the surface of platelets of patients with panmyelopathy among them the present patient but not at platelets of normal people

We have to keep in mind various possible explanations for this phenomenon The first is that these platelet associated antibodies play a causative role in the pathogenesis of the bone marrow damage Another possibility is that they are not responsible for the myelopathy and should be regarded only as a possible indication of an immunological mechanism Recent experimental studies yielded in vitro evidence of a cell mediated immunological mechanism in a case of panmyelopathy (aplastic anaemia) (1 9)

Antibodies active in inflicting bone marrow damage in the mother might pass through the placenta The newborns of mothers with blood disorders such as thrombocytopenia mediated by antibodies (ITP) may temporarily exhibit the same condition by the passive transfer of these antibodies In the present case as well as in other infants born to mothers with panmyelopathy there were no definite signs of bone marrow damage In our opinion it seems unlikely that the minor degree of leukopenia and thrombocytopenia on the first day was due to insufficient production of blood cells Likewise we have not been able to detect antibodies affecting platelets in the presence of PCMBS in the serum of the newborn The technique for detecting platelet membrane associated immunoglobulins was not available when the child was born These immunoglobulins however might be incapable of

passing through the placenta because they are part of immune complexes adsorbed on the platelet surface

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Intracutaneous Herniation of Fat in Connection with Microangiopathia Diabetica

Kurt Einarsson Stig Hård Barbro Leijd and Emil Neumann

From the Department of Dermatology St Goran s Hospital and the Department of Medicine Serafimerlasarettet Karolinska Institutet Stockholm Sweden

ABSTRACT Three women with diabetes mellitus presented an unusual change in the skin on the distal and acral parts of the extremities, most distinctly on the upper surface of the hands The surface was raised in small cushions but had the colour of intact skin The skin felt doughy and swollen on palpation but there was no oedematous pitting It had been suspected earlier that these skin changes indicated myxoedema or heart disease and the patients had been treated with diuretics In all three cases how ever, histological examinations revealed a more or less reduced, atrophic dermis with here and there an appreciable dislocation of the subcutaneous layer of fat, which with the sweat glands formed an in tracutaneous herniation up against the epidermis These histological changes explained the clinical picture of cushions in the skin As these patients had diabetes mellitus and the histological picture also in cluded vascular changes of the type associated with a diabetic microangiopathy, it is considered that this form of intradermal herniation of fat is yet another skin change which may be elicited by diabetes mel litus

Herniation of fat in the skin of the eyelid was sug gested by Elschnig (4) in the 1930s as the cause of chronic baggy lower eyelids Many years later Parkes and Griffiths (10) demonstrated that this condition arose when the intraocular fat tissue protruded through a weakened or defective septum orbitale into the subcutaneous tissue in the lower eyelid Mini hernias can thus be formed in the skin by the penetration of fat from adjacent areas The dislocated fat tissue may have a capsule of connec tive tissue (7) and its own blood vessels (9) Soli tary protrusions around crena ani which used to be interpreted as lipoma have proved to be due to herniation of fat (9) Such herniation also occurs on an even smaller scale In some cases the protrusion of subcutaneous fat into the cutis cannot be de tected without a microscope Weakened connective tissue may be primary cause though pressure and trauma may be the eliciting factor These in tracutaneous herniations have been studied chiefly in connection with—and have been regarded as the cause of—what are known as painful piezogenic papules (3 7 11 12 14) and the soft cushioning of the skin in Goltz–Gorlin s syndrome (2)

We have observed uneven sponge and cushion like elevations of the skin, particularly on the dorsa of the hands in three patients with diabetes mel litus In each case microscopy showed subcutane ous herniation of fat into the dermis

CASE REPORTS

Case 1

A woman of 52 Since 1968 diabetes mellitus requiring increasing doses of insulin for the past three years Dis comfort from angina pectoris and cardiac decompensation for the past 2 years Admitted in 1974 to the Department of Medicine on account of breathlessness and poorly control led diabetes She presented as an overweight woman with a pasty skin reminiscent of myxoedema and a suggestion of cushingoid features which on two previous occasions had led to endocrinological studies with myxoedema and Cushing s disease in mind but with negative results

For at least one year there had been an uneven doughy swelling of the skin on the upper surface of the feet and hands particularly the latter and to a lesser extent in a 10 cm long area on the distal parts of the forearms and lower legs No reddening or temperature increase of the skin which had the colour of intact skin and was not tender upon palpation neither did the patient report itching or pain Upon palpation there was none of the pitting as sociated with oedema but the skin felt spongy and quickly returned to its original configuration after pressure or pinching The cushion like protuberances of the hands are seen in Fig 1



Fig 1 Cushion-like protruberances of the skin on the upper surface of the hands (case 1)



Fig 3 Pas-positive fibrillar thickening and protrusion of endothelial cells in blood vessel walls most emphasized to the right with some neocapillaries in the vicinity Ramification and fragmentation of collagen fibres

Routine laboratory tests showed normal values except for serum creatinine (1 5 mg/100 ml) Thyroid tests gave normal values Cholesterol 400 mg/100 ml triglycerides 5 1 mmol/l Heart-lung X rays showed a somewhat enlarged heart but no pulmonary changes

Treatment with a suitable weight reducing diet reduced the insulin requirement to 36 IU Insulin Novo Semilente® a day Other treatment Lanacrist® (digoxin) Lasix® (furosemide) Kalitabs® (potassium chloride) Sensaval® (nortriptyline chloride) and Pacinol® (fluphentiazine chloride)

Histological examination Punch biopsies were taken from the skin on the back of the hand and the lower leg fixed in 10% formalin and stained routinely with haematoxylin-eosin Van Gieson and with Weigert s elastic stain PAS Alcin blue and iron.

croscopy showed the same changes in the hand and t they were most pronounced in the former The is was not appreciably changed The corium was ly atrophic in parts being grossly reduced in some places and there was a striking dislocation of fat and



Fig 2 Fat and sweat glands displaced to the upper part of the corium Atrophy of the collagen

sweat glands these being located high up into the corium near epidermis (Fig 2) In these areas there were short thin partly granulated collagen fibres arranged irregularly and split up with large gaps in between Elastic fibres were profuse and thickened partly clumped together and fragmented particularly around the fat protruding into the cutis and around the sweat glands The small and medium sized blood vessels had grossly thickened walls containing intensely PAS-positive fibrillar material The endothelium was swollen with knobby indentations unevenly stained and defective in places (Fig 3)

Cases 2 and 3

Women 77 and 76 years of age They were clinically and histologically identical and resembled case 1 Both had had diabetes for several years and since a couple of years cushioning of the skin on the dorsum of the hands and fingers like case 1 Case 2 had cushion like consistency of the skin on the legs Case 3 had atrophic maculae a few mm large scattered on the legs Both ladies had been subjected to a hormonal investigation because of the appearance of the skin however with negative results except for their diabetes

Punch biopsies were taken from their hands and legs In specimens from the hands the picture was like that in case 1 with sweat glands dislocated in fat tissue which penetrated into the dermis towards the surface layer The walls of small and medium-sized vessels were thickened and displayed intensely PAS-positive fibrillar material indicating diabetic angiopathy Such vessels were also found in the skin from the legs of case 2 but not of case 3 who only had epidermal atrophy in the above mentioned maculae

DISCUSSION

The present three patients with cushioning of the skin on the upper side of the hands and in two cases with indications of similar changes on the

legs were all women though this could be a coincidence It is noteworthy that they all had diabetes mellitus They were all over 50 two of them even over 70 years old The histological picture was largely uniform in all three cases The microscopic picture was dominated by dislocation of the subcutaneous fat layer into the corium where it lied in places with the sweat glands near the epidermis This dislocation of the subcutaneous fat must be attributed to a degeneration of the collagen fibres The increase of the substance which absorbed elastica staining was very probably relative even though the visual effect suggested an absolute rise The microscopic examination also showed that the elastic fibres in the corium were fragmented that the collagen fibres were thinner and ran a more irregular course than normal and that the dislocated fat tissue was embedded in the corium without a capsule

These histological changes could well explain the unusual changes in the skin which felt spongy upon palpation with cushion like protruberances We do not assert that these skin changes are definitely due to the diabetic disorder Having found them only in patients with diabetes however we suspect that this disease is one basic cause of such changes appearing in the skin

Lipodystrophy and other skin changes in diabetes treated with insulin are well known (1 5 8) and arise as a rule in the vicinity of the insulin depots In the present patients the skin changes had no connection with the location of insulin injections Elastosis has been described in patients with chronic acidosis (6 13) but our patients were not and never had been in such a state The degenerative changes in the collagen and elastic fibres are most probably caused primarily by the vascular changes which represent a diabetic microangiopathy It is conceivable that the vascular changes lead to nutritional and chemical disturbances in the corium that weaken it and result in the herniation of the fatty tissue up and into the corium We presume that the dislocation of the subcutaneous fat in our patients was a consequence of their diabetic microangiopathy Our review of the literature to date suggests that such clinical characteristic skin changes have not been demonstrated before in conjunction with diabetes mellitus

The skin changes in our patients had caused some diagnostic and therapeutic problems All three patients had undergone an endocrinological examination for suspected myxoedema on one or several occasions but thyroid function was alwasy found to be normal The skin changes had been interpreted by several physicians as a more or less chronic oedema and all three women had accordingly been treated with diuretics without intended effect We therefore recommend that in patients who present unusual spongy cushioning of the skin of the hands and feet a skin biopsy should be taken as in our experience this may save both patients and physicians from going through lengthy examinations and attempts at therapy

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ANNOUNCEMENT

International Congress of Inflammation will be held in Bologna Italy Oct 31–Nov 4 1978 and a satellite Symposium of this Congress *Pain and Inflammation* Nov 4–5 1978 The working languages of the Congress and Symposium will be English and Italian

Information Organizing Secretariat Dr E Folco FGL Via Monte Napoleone 23 I 20121 Milan Italy

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Treatment of Diuretic-Resistant Fluid Retention with Ultrafiltration

H Asaba J Bergstrom P Furst S Shaldon and S Wiklund

From the Department of Nephrology and the Metabolic Research Laboratory St Erik s Hospital Stockholm Sweden

ABSTRACT Nine patients with diuretic resistant edema, secondary to congestive heart failure liver cirrhosis, or nephrotic syndrome, were treated with ultrafiltration using high water flux dialyzers Access to the blood stream was obtained by femoral vein catheterization As much as 8 3 kg of fluid were removed in 3–4 hours with only transient decline in blood pressure The procedure was well tolerated and yielded immediate symptomatic relief The potential for restoration of an edema free state in patients with diuretic resistant edema suggests that further experience with this technique is justified

Severe fluid retention still presents a therapeutic problem whether the cause is congestive heart failure liver cirrhosis or nephrotic syndrome Although we now have efficient diuretics such as furosemide or ethacrynic acid some patients do not respond to such therapy

Silverstein et al (16) suggested in 1974 that such patients might be treated using an ultrafiltration unit to remove the excess plasma water and sodium Following the observations (1 2 6) that removing fluid by ultrafiltration alone was well tolerated in overhydrated dialysis patients we applied this technique to the treatment of non-dialysis patients with diuretic resistant fluid retention (14) Recently other groups have also reported favorable results with ultrafiltration in overhydrated non uremic patients (4 9 12)

PATIENTS MATERIAL AND METHODS

Nine patients suffering from a variety of primary diseases with fluid retention resistant to diuretics were treated (Table I) Informed consent was obtained from all The three patients with primary heart disease had only a slight reduction of renal function whereas the others had more advanced renal failure (Table II) None of them was considered to require dialysis for other reasons than fluid overload since they did not exhibit any clinical signs of uremic toxicity All patients had shown resistance to intensive diuretic therapy in hospital

The ultrafiltration units used were either the Rhone Poulenc RP-6 dialyzer or the Gambro Ultradiffuser which are commercially available artificial kidneys with high ultrafiltration capacities

The RP-6 unit has a surface area of 1 03 m^2 arranged in 16 parallel layers The membrane is made of polyacrylonitrile 30 μm thick which is highly permeable to water and has a molecular cut-off at 40000 daltons It is impermeable to albumin (11) The Gambro Ultradiffuser has a surface area of 1 86 m^2 arranged in 28 parallel layers with an 11 5 μm cuprophane membrane highly permeable to water and a molecular cut-off at 5000 daltons (13) The dialysate compartment was closed at one port and the other was used as ultrafiltrate outlet (Fig 1)

The extracorporeal circuit was prepared using a standard hemodialysis blood tubing set (Gambro sterilized disposable blood lines A155A8 and V153A) and primed with heparinized saline solution Access to the blood stream was obtained by bilateral percutaneous femoral vein catheterization (15) in six patients The blood circulation through the ultrafiltration unit was maintained by a roller blood pump (Gambro Blood Pump BP 3A) and the flow rate was kept at about 200–300 ml/min In three patients (nos 1 8 and 9) single needle technique (8) was applied where only one catheter was inserted in a femoral vein and used for to and fro blood access using a single needle machine (Gambro-SNM) (10)

Heparinization was carried out by an i v priming dose of 5000 IU followed by a continuous infusion of 500–750 IU/hour

The desired transmembrane pressure gradient was produced either with a screw clip placed on the blood tubing in the down stream of the air bubble trap or with a negative pressure pump (Gambro Blood Pump BP 3A) attached to the ultrafiltrate outlet (Fig 1) The ultrafiltrate was collected into a measuring cylinder either through the ultrafiltrate outlet of the unit or through the negative pressure pump The accumulated volume of the ultrafiltrate was readily checked by looking at the fluid level of the measuring cylinder

[1] *Present address* Rue d Alco F 34000 Montpellier France

Table I *Clinical data on the patients*

Pat no	Age (y)	Sex	Etiology of fluid retention	Signs of fluid retention
1	63	♂	Congestive heart failure (mitral valve prosthesis)	Cardiomegaly hepatomegaly peripheral edema
2	56	♂	Nephrotic syndrome (diabetes)	Cardiomegaly pleural effusion ascites peripheral edema
3	41	♀	Nephrotic syndrome (diabetes)	Cardiomegaly pleural effusion ascites peripheral edema
4	38	♀	Nephrotic syndrome (diabetes)	Pleural effusion cardiomegaly ascites peripheral edema
5	66	♂	Congestive heart failure (arteriosclerotic heart disease)	Pulmonary edema cardiomegaly peripheral edema
6	63	♂	Nephrotic syndrome (amyloidosis)	Pleural effusion cardiomegaly hepatomegaly ascites peripheral edema
7	65	♂	Liver cirrhosis	Ascites peripheral edema
8	63	♀	Nephrotic syndrome (diabetes)	Cardiomegaly pulmonary edema peripheral edema
9	57	♀	Congestive heart failure (mitral insufficiency)	Cardiomegaly peripheral edema

BP and pulse rate were measured every 15 min throughout the procedure and even more frequently when necessary Blood samples for hematocrit plasma sodium potassium osmolality total protein and creatinine were taken at the start and at the end of the procedure

Sodium and potassium concentrations were expressed as concentrations in plasma water (Table III) which was calculated from the total protein concentration by applying the following equation

$H_2O_p = 984 - 0\ 718 \times$ protein concentration (g/l) (5)

RESULTS

e volume of ultrafiltrate removed in the 9 pa ts averaged 5 5 l The mean duration of the procedure was 183 min This represents a mean filtration rate of 30 ml/min (Table III) This was associated with an average increase in hematocrit of 5 8% and in plasma protein of 16 6 g/l There were no significant changes in the concentration of potassium or creatinine in plasma water or in total plasma osmolality during ultrafiltration except for patient 1 whose posttreatment plasma samples were hemolyzed Sodium concentration in plasma water increased by 2 7 mmol/l probably due to the infusion of sodium chloride (Table II) In all patients a decrease in peripheral edema was observed and in patients with pulmonary edema the relief of

Table II *Biochemical data*

Pat no	Hematocrit (%)		Creatinine (μmol/l)	Protein (g/l)		Na^+ (mmol/l)		K^+ (mmol/l)		Osmolality (mOsm/kg)	
	Start	End	Start	Start	End	Start	End	Start	End	Start	End
1	47	56	119	67	95	149	151	3 9	4.5	280	281
	35	50	119	64	94	149	149	3 9	4 6	280	280
	37	42	132	66	93	146	153	3 4	3 9*	280	281
	42	42	130	56	65	143	143	4 1	4 1	285	280
2	39	41	739	61	67	146	147	4 0	4 1	290	301
3	27	37	337	57	76	144	146	3 4	3 8	291	289
	25	32	255	42	72	139	141	3 2	3 3	302	301
4	27	32	447	–	–	–	–	–	–	–	–
5	43	47	180	56	68	143	142	5 4	5 1	297	292
6	17	21	–	28	41	147	155	3 8	4 1	–	–
7	–	–	650	56	64	131	136	4 0	4 4	281	284
8	33	40	640	36	50	149	152	3 1	3 6	–	–
9	42	43	170	41	44	134	137	4 0	3 9	360	360

* Blood samples were hemolyzed

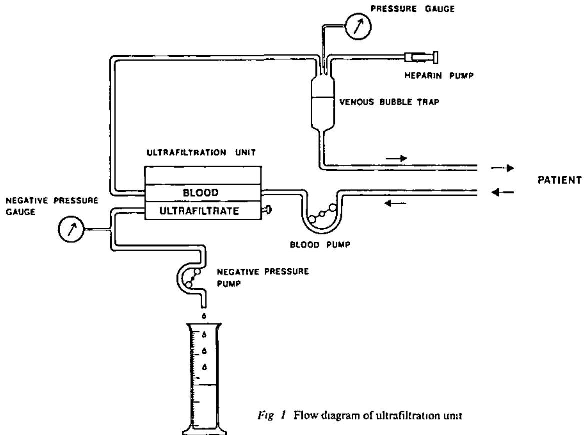


Fig 1 Flow diagram of ultrafiltration unit

dyspnea was remarkable The reduction of pleural effusion and ascites in the other patients resulted in symptomatic improvements

Although the procedure was well tolerated a transient fall in BP occurred when ultrafiltration was too rapid Hypotension was remedied by interrupting ultrafiltration by clamping the ultrafiltrate outlet tube or stopping the negative pressure pump This usually resulted in a spontaneous rise in BP within 15 min Intravenous albumin 20 g in 100 ml was successfully given to patients 6 and 8 to treat hypotension Three patients experienced leg muscle cramps which responded to infusion of 10-15 ml of 4 M hypertonic sodium chloride solution (7)

DISCUSSION

Diuretic resistant fluid retention is a most distressing situation to the patient and presents a difficult therapeutic problem The amount of excess fluids removed by venesection or scarification is limited and often not sufficient Peritoneal dialysis has also been tried with some success Unfortunately the rate of fluid removal is low and protein is lost in the dialysate which may cause hypoproteinemia and increase the edema in the peripheral tissues

The ultrafiltration was effective in removing excess fluids and was remarkably well tolerated with only an occasional transient fall in BP which was readily remedied This is surprising in view of the rapid removal of fluid from the vascular compartment

In dialysis patients hypovolemia with decrease in cardiac output induced by ultrafiltration (with concomitant dialysis) is compensated for by an
crease in peripheral vascular resis (3)
stable BP is maintained prov e rat
ultrafiltration is not excessive tion
crease in plasma protein con cau
ultrafiltration mobilizes the m ede
tissue to the intravascu men
pensating f volem

Table III *Pretreatment body weight treatment time weight loss ultrafiltration rate and blood pressure*

Pat no	B wt (kg)	Duration of treatment (min)	Total weight loss during treatment (kg)	Average ultra filtration rate (ml/min)	BP (mmHg)	
					Start	End
1	77 1	140	3 3	24	110/80	110/80
	75 8	220	6 5	30	120/75	115/80
	74 9	175	5 2	30	115/60	110/60
	75 2	185	4 5	22	115/70	115/80
2	88 4	120	4 2	35	130/90	125/85
3	53 7	200	8 2	41	170/108	120/100
	58 5	210	7 8	37	160/90	130/90
4	69 8	210	8 3	40	200/95	110/80
5	45 0	95	2 0	21	60/45	62/44
6	70 0	270	8 1	30	170/87	117/63
7	87 0	215	4 5	21	65/40	50/40
8	75 0	210	5 5	26	170/90	130/70
9	55 8	105	3 2	30	140/40	140/80

Single needle technique often used in routine *hemodialysis has been applied in order to simplify* vascular access This technique combined with a negative pressure pump on the ultrafiltrate side *functioned well hemolysis occurred however* when it was used with positive pressure in the blood compartment created by a screw clip on the blood line

An interesting observation was that 2 patients [illegible] 1 and 6) regained responsiveness to furosemide following ultrafiltration In patient 1 edema continued to subside after a first series of treatments and 7 months elapsed before the next treatment became necessary Patient 6 has been treated only once after which the edema subsided further with furosemide the patient is still edema free 7 months after the ultrafiltration

It thus appears that ultrafiltration may be a promising alternative to other treatments for diuretic resistant fluid overload In the hands of a routine dialysis staff the technique is simple and safe The ultrafiltration device is easily set up in a patient room on the ward using readily available equipment and materials from a dialysis unit Further evaluation of this treatment on a long term basis seems justified

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Influence of Sodium Intake on Hydrochlorothiazide-Induced Changes in Blood Pressure, Serum Electrolytes, Renin and Aldosterone in Essential Hypertension

P van Brummelen M Schalekamp and J de Graeff

From the Department of Nephrology University Hospital Leiden and the Department of Internal Medicine Erasmus University Rotterdam The Netherlands

ABSTRACT To determine the influence of dietary sodium intake on the effects of hydrochlorothiazide (HCT) on blood pressure (BP) serum electrolytes, renin and aldosterone, nine male patients with uncomplicated essential hypertension were studied during the following therapeutic regimes 1) sodium restriction alone (50 mmol/day) 2) sodium restriction combined with HCT (50 mg twice daily), 3) HCT alone and 4) sodium restriction combined with HCT Low sodium diet alone and HCT alone lowered BP to the same extent The combination of HCT and sodium restriction had no extra effect on supine BP, but elicited complaints of dizziness and weakness in each patient, and overt orthostatic hypotension in three cases Sodium restriction during HCT treatment caused hyponatraemia and aggravated hypokalaemia Hyponatraemia could not be accounted for solely by changes in cumulative sodium balance Plasma renin concentration rose markedly during the combined treatment Plasma aldosterone was normal during HCT alone but elevated when HCT was combined with sodium restriction These results cast some doubt on the therapeutic value of prescribing a low sodium diet to patients with essential hypertension treated with thiazide diuretics Overactivity of the renin–angiotensin–aldosterone system during this regime might explain both the lack of a beneficial effect on BP and the adverse influence on serum potassium

Thiazide diuretics are widely used in the treatment of hypertension Although most investigators agree that the antihypertensive effect of these drugs is somehow related to the urinary loss of sodium (7 19 35 37) the efficacy of dietary sodium restriction during thiazide treatment has not been extensively studied and is subject to controversy (8 21 22 27) Moreover pertinent data on the metabolic consequences of combined thiazide therapy and salt restriction are lacking Therefore we studied the influence of sodium intake on BP serum electrolytes and the plasma concentrations of renin and aldosterone in patients with uncomplicated essential hypertension treated with hydrochlorothiazide

PATIENTS

Nine patients (male age 20–50 years) were studied both under standardized conditions in a metabolic ward and as outpatients A diagnosis of essential hypertension was made after a search for secondary causes using investigations that included excretory urography radioisotope renography and urinary excretion of vanillylmandelic acid Serum creatinine was normal in all patients (less than 120 μmol/l) Three cases showed ECG signs of left ventricular hyperthophy (SV_1+RV_5>3 5 mV) Eye ground changes were of grade I or II

STUDY PROTOCOL

In the first study period patients were seen weekly in the Outpatient Clinic for a period of four weeks At each visit BP (supine and standing pulse rate and body weight were measured and blood was drawn for determination of serum electrolytes Patients had no medication and dietary sodium intake was not restricted Urinary sodium excretion varied between 150 and 200 mmol/day

After this period patients were admitted to the metabolic ward and were put on a fixed diet containing 50 mmol of sodium and 90 mmol of potassium per day Sodium excretion was measured daily BP and pulse rate were measured daily at 11 a m and 4 p m Hydrochlorothiazide (HCT) 50 mg twice daily was given after nine days for a period of eight days On the day before HCT was started and after seven days of treatment blood samples for determination of plasma renin concentration (PRC) and plasma aldsterone (PA) were taken at 9 a m after at least ten hours of recumbency Blood for determination of serum electrolytes was taken at regular intervals

Abbreviations HCT=hydrochlorothiazide BP=blood pressure PRC=plasma renin concentration PA=plasma aldosterone

Table I Blood pressure (mmHg) pulse rate (PR beats/min) and body weight (kg) before treatment during sodium restriction and during hydrochlorothiazide with and without sodium restriction

sup=supine st=standing

Case no		Without HCT						During HCT (50 mg twice daily)					
		Sodium intake (mmol)						Sodium intake (mmol)					
		150–200			50			50			150–200		
		BP	PR	B wt.	BP	PR	B wt	BP	PR	B wt	BP	PR	B wt
1	sup	168/101	80	61 0	159/104	81	61 0	159/105	89	59 7	153/103	84	60 1
	st	166/111	92		161/109	95		120/88	116		136/101	100	
2	sup	172/112	57	75 0	132/97	61	74 0	142/91	75	72 5	149/99	66	73 7
	st	179/113	55		134/89	74		150/98	102		149/99	75	
3	sup	168/116	63	77 9	134/96	69	74 9	124/98	85	72 3	136/89	75	78 6
	st	169/117	84		117/91	119		92/84	138		126/92	111	
4	sup	147/102	68	74 8	146/104	65	74 5	142/106	72	70 8	138/99	72	75 6
	st	150/116	86		153/119	79		143/111	96		144/111	87	
5	sup	140/99	75	82 7	121/91	76	82 1	103/83	84	79 3	124/91	81	83 2
	st	139/109	85		130/109	96		97/85	127		127/106	105	
6	sup	142/95	67	80 3	133/79	60	80 8	137/92	70	78 4	137/92	67	82 4
	st	150/105	77		141/99	67		122/97	92		142/106	78	
7	sup	137/92	69	84 7	127/87	73	83 0	128/90	70	81 9	136/96	69	83 2
	st	154/110	76		141/103	89		140/105	98		148/111	81	
8*	sup	174/117	77	106 7	170/111	75	104 1	163/109	80	101 2	163/107	73	88 1
	st	190/138	83		180/131	90		161/134	102		170/127	85	
9	sup	164/122	77	85 6	137/95	62	83 8	137/99	75	80 5	142/102	75	83 9
	st	166/130	78		135/103	73		118/95	105		143/112	88	
Mean	sup	156/106	70	77 7	140/96	69	76 8	137/97	77	74 4	142/98	74	77 6
	st	163/117	80		144/106	87		127/100	108		143/107	90	

* Body weight not used for calculating mean value because of deliberate weight loss

During a subsequent period of four months patients were biweekly in the Outpatient Clinic while HCT treat was continued Dietary sodium intake at that time not restricted Again daily sodium excretion varied 150 and 200 mmol Halfway through this period blood samples for determination of PRC and PA were taken at noon after three hours of recumbency

Finally the patients were readmitted to the metabolic ward for a period of nine days Again a fixed diet containing 50 mmol of sodium and 90 mmol of potassium per day was given while HCT was continued Measurements of BP pulse rate serum electrolytes sodium excretion PRC and PA were repeated

METHODS

BP was measured with a mercury sphygmomanometer after 10 min supine rest and 1 min standing Pulse rate was recorded at the same time Phase IV Korotkoff sounds were taken as diastolic BP BP and pulse rate figures used for subsequent analysis were 1) the mean of the values during the last two visits to the Outpatient Clinic and 2) the mean of the values during the last two days of each study period in the metabolic ward

PRC was determined by radioimmunoassay (29 32) after processing the plasma according to Skinner (30) PA was determined in the laboratory of M Frolich by radioimmunoassay as described by Bayard et al (?) and Frolich et al (11)

Student s t test for paired observations was used to determine statistical significance of differences Values of $p<0.05$ were regarded as significant

RESULTS

BP pulse rate and body weight

Individual data are summarized in Table I Mean values ±S E M in each study period are shown in Fig 1 and levels of significance for differences in Table II

Admission to the metabolic ward with the institution of a low sodium diet lowered BP significantly *A similar fall in BP was observed after four months* of treatment with HTC alone Dietary sodium restriction during diuretic treatment caused a distinct fall in body weight but had little additional effect on supine BP Standing BP was slightly but significantly reduced This reduction in BP was most pro-

0		
P	PR	B wt
50/100	90	60 7
26/95	111	
45/97	73	72 7
35/94	104	
127/86	85	75 1
06/87	134	
38/102	65	72 5
142/112	93	
111/87	77	81 2
108/94	110	
142/91	65	79 4
134/101	87	
124/87	70	81 8
135/103	94	
141/93	75	83 6
140/114	99	
127/91	62	81 7
120/97	85	
134/93	74	75 6
131/100	102	

nounced for the systolic pressure and was accompanied by a marked increase in pulse rate In fact the reduction of the mean value of standing BP for the whole group was due to the occurrence of serious hypotension in three patients

Sodium potassium renin and aldosterone

Individual data are given in Table III Mean values ±S E M in each study period are shown in Fig 1 and levels of significance for differences in Table II Serum sodium was normal during HCT treatment without sodium restriction When the diuretic was combined with a low sodium diet serum sodium was significantly reduced HCT induced hypokalaemia was aggravated by dietary sodium restriction the differences being highly significant ($p<0\ 001$) HCT with or without sodium restriction increased PRC compared with values obtained during sodium restriction alone As expected PRC was highest when HCT was combined with sodium restriction Again the difference was statistically significant ($p<0\ 001$) During this regime also PA was significantly higher than during HCT alone ($p<0\ 005$) Log PRC and PA in the different study periods were significantly correlated ($r=0\ 55$ $p<0\ 001$) During HCT without sodium restriction the ratio PA to log PRC was 7 8±2 2 (mean ±S E M) when sodium was restricted it was 17 1±1 6 This difference was statistically significant ($p<0\ 005$)

Cumulative sodium balance

Cumulative sodium balance was calculated in both periods with HCT and a low sodium diet (Fig 2) After the first eight days on HCT cumulative sodium balance was negative in seven patients and positive in two (mean −102 mmol range −404 to +29) At the end of the second clinical period after four months of treatment, cumulative sodium balance was positive in six out of nine patients (mean +47 mmol range −89 to +221)

DISCUSSION

It is well established that the antihypertensive effect of thiazide diuretics can be abolished by intake of large amounts of salt (10 38) One would therefore expect the BP lowering effect of these agents to be enhanced by dietary sodium restriction On this ground many physicians prescribe low sodium diets to their hypertensive patients when they are on thiazide diuretics but surprisingly enough until now there are little data either to support or to refute this therapeutic policy

Kirkendall and Overturf (22) reported on a drop of BP in four out of six patients with hypertension treated with chlorothiazide when salt intake was reduced from 12 to 4 g/day

Parijs et al (27) using the combination of HCT and spironolactone observed a significant albeit small fall of BP when dietary sodium intake was reduced However in their study sodium intake was not standardized

In the present study the BP lowering effect of sodium restriction (50 mmol/day) alone was compared with the effect of HCT alone and in combination with sodium restriction Both a low sodium diet alone and HCT alone lowered the BP Sodium restriction during HCT had no extra effect on the mean value of systolic and diastolic supine BP upright BP however fell significantly Individual pressure responses varied from overt orthostatic

Table II *P values for paired differences in BP pulse rate body weight sodium potassium renin and aldosterone between the study period without treatment and all subsequent periods*

PRC and PA are compared with values obtained during sodium restriction

	No HCT 50 mmol sodium	During HCT (50 mg twice daily) Sodium (mmol) 50	150–200	50
BP				
Systolic supine	<0 02	<0 01	<0 005	<0 005
Diastolic supine	<0 02	<0 05	<0 05	<0 02
Systolic standing	<0 02	<0 001	<0 005	<0 001
Diastolic standing	<0 05	<0 005	<0 02	<0 005
Pulse rate				
Supine	N S	<0 05	N S	N S
Standing	N S	<0 001	<0 02	<0 005
Body weight	<0 05	<0 001	N S	<0 005
Serum sodium	<0 05	<0 001	N S	<0 01
Serum potassium	<0 02	<0 001	<0 001	<0 001
PRC		<0 001	<0 01	<0 001
PA		<0 005	N S	N S

N S – not significant

hypotension to no change at all All cases showed considerable orthostatic tachycardia and had more or less serious complaints of dizziness weakness and anorexia when HCT treatment was combined with a low sodium diet

The reduction in systolic and diastolic supine and upright BP levels obtained after four months of HCT alone were directly correlated with the effects of sodium restriction alone ($n=36$ $r=0\ 80$ $p<0\ 001$) This is in keeping with the view that both types of treatment have a similar mode of action which is somehow mediated by their effects on so-

Table III *Levels of serum sodium (mmol/l) and potassium (mmol/l) plasma renin concentration (ng/ml/h) and plasma aldosterone (ng/100 ml) before treatment during sodium restriction and during hydrochlorothiazide with and without sodium restriction*

Case no	Without HCT Sodium intake (mmol) 150–200* Na	K	50 Na	K	PRC	PA	During HCT (50 mg twice daily) Sodium intake (mmol) 50 Na	K	PRC	PA	150–200 Na	K	PRC	PA
1	141	4 0	139	4 4	22 9	21 8	135	3 1	82 0	46 0	139	3 3	31 4	6 8
2	141	4 5	139	4 8	12 5	15 0	136	3 1	88 3	33 0	139	3 6	21 8	18 4
3	143	4 0	137	4 3	12 0	1 9	133	3 0	91 5	36 0	140	3 7	25 2	4 7
4	144	4 0	140	4 0	8 4	21 0	135	2 6	37 2	37 5	140	3 3	6 9	17 0
5	141	4 0	140	4 4	18 0	20 0	133	2 7	97 8	34 0	138	3 1	39 6	15 0
6	141	3 8	139	4 0	9 0	14 0	136	2 7	49 4	53 0	140	3 5	15 4	2 5
7[b]	139	4 0	140	4 1	10 4	8 2	135	3 1	29 9	11 6	140	3 5	—	—
8	140	3.8	139	3 8	9 0	11 6	140	2 5	43 4	11 5	140	2 8	15 0	4 5
9	140	3 9	140	3 9	7 5	6 7	137	2 9	55 8	20 0	141	3 0	32 4	8 0
Mean	141	4 0	139	4 2	12 4	14 0	136	2 9	67 7	33 9	140	3 3	23 5	9 6

* PRC and PA not measured in this study period
[b] PRC and PA values not used for calculating mean values

dium balance (6 7 27) The finding that supine BP during treatment with combined HCT and sodium restriction was not significantly lower than during either HCT or sodium restriction alone might be related to the higher plasma levels of renin and aldosterone observed during the combined treatment Direct evidence for an important role of the renin–angiotensin system in BP control during dietary sodium restriction and diuretic treatment has been provided by clinical and experimental studies with the specific angiotensin II antagonist saralasin (13 18 31 33) Increased sympathetic nervous activity as shown by the tachycardia in the upright position will also counteract the BP lowering effect of diuretics

Serum sodium has been reported to be normal during treatment of essential hypertension with thiazide diuretics by some authors (14 16) while others (9 28) observed modest or even considerable hyponatraemia In our study serum sodium did not differ from baseline values when HCT was given without sodium restriction However serum sodium was significantly lowered when HCT was combined with a low sodium diet Our data showed a positive cumulative sodium balance in two out of nine patients on the eighth day of the first period of combined treatment with HCT and sodium restriction During the second period of this combined treatment the mean cumulative sodium balance of

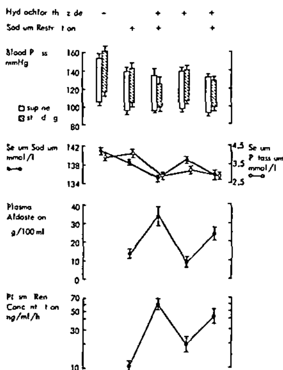


Fig 1 Mean values (± S E M) for BP serum sodium serum potassium PA and PRC before treatment during sodium restriction and during HCT with and without sodium restriction

nine patients was positive (Fig 2) Therefore decreased serum sodium could not be accounted for solely by urinary sodium loss Several other mechanisms might be involved Possibly as an consequence of volume depletion ADH is increased (9) Potassium depletion may cause a sodium shift to the cells (9 24) and interference with the renal diluting mechanism by thiazide diuretics could also play a role (1 15)

Hypokalaemia is a well known consequence of thiazide therapy (14 25 34) It has been suggested that a high sodium intake during treatment with thiazide diuretics would increase hypokalaemia by enhancing urinary potassium loss (20 23) *However the present study showed that unrestricted* sodium intake during treatment with HCT did not aggravate hypokalaemia On the contrary serum potassium was significantly higher than when HCT was combined with a low sodium diet It is unlikely that the decrease in serum potassium by sodium restriction was caused by a diminished intake of potassium since the diet contained 90 mmol of potassium per day which is not

50

Na	K	PRC	PA
135	3 2	44 0	25 0
134	3 4	67 1	22 0
135	3 3	40 9	34 0
137	2 7	31 5	24 5
134	2 7	107 0	14 8
139	3 1	29 8	33 0
138	3 5	20 3	35 0
142	2 6	20 6	10 0
138	2 9	54 4	37 0
137	3 0	49 4	25 0

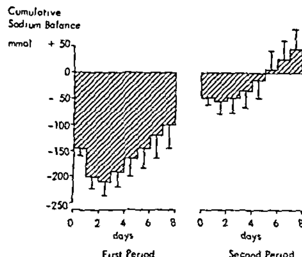


Fig 2 Mean cumulative sodium balance (± S E M) during combined HCT and sodium restriction at the beginning of HCT treatment (first period) and after 4 months of treatment (second period)

less than in a regular diet Presumably the observed changes in PRC and PA are to be invoked here Both renin and aldosterone were clearly elevated when HCT was combined with a low sodium diet but in contrast to renin aldosterone was normal when sodium intake was not restricted Thus secondary aldosteronism during thiazide therapy only found when sodium intake was restricted aldosterone excretion or plasma aldo- ıe during long term treatment with HCT has reported by several authors (4 14)

The observed increase in the ratio PA to log PRC during sodium restriction suggests that other factors than the renin–angiotensin system may contribute to the hyperaldosteronism which occurs when HCT is combined with a low sodium diet Hyponatraemia (3 5) diminished metabolic clearance rate of aldosterone (12 39) and increased sensitivity of the adrenal cortex for angiotensin II (17 26 36) could play a role here

In conclusion the present study which admittedly involves a small number of patients does not support the view that dietary sodium restriction to 50 mmol/day is of any benefit during treatment of essential hypertension with HCT

ACKNOWLEDGEMENT

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Hypertensive Eye Ground Changes

Prevalence Relation to Blood Pressure and Prognostic Importance
The Study of Men Born in 1913

Kurt Svardsudd Hans Wedel Elisabeth Aurell and Gösta Tibblin

From the Section for Preventive Cardiology Medical Department I Sahlgren s Hospital Gothenburg Sweden

ABSTRACT As part of a prospective population study of cardiovascular disease, ophthalmoscopic examination was performed in 855 randomly selected 50-year-old men in 1963 The examination was repeated four years later, in 1967 On both occasions haemorrhages exudates and papillary oedema were rarely seen Attenuating arterioles, focal narrowing crossing phenomena and broadened light reflex were all related to BP but attenuating arterioles and/or focal narrowing discriminated BP best Isolated broadened reflex and/or crossing phenomena were not related to BP The change in BP over four and ten years was the same in those who had and those who did not have hypertensive eye ground changes at the initial examination, indicating that eye ground changes do not precede hypertension Mortality data and the morbidity in myocardial infarction and stroke were followed up until 1975 The importance of the separate eye ground variables for these mortality and morbidity end points was analyzed, taking into account the importance of BP, serum cholesterol and smoking Focal narrowings were of importance for mortality regardless of its cause for stroke and for death from malignancy Crossing phenomena were of importance for mortality, regardless of cause, for fatal coronary heart disease for stroke and for deaths other than cardiovascular and cancer deaths The eye ground variables are thus of differing importance for different end points Grouping them as in Keith and Wagener s or other classification systems, means a loss of information and should be avoided

All hypertensive ophthalmoscopic findings known to-day were described before 1900 Since then little new has been added to the description in spite of the vast literature on the subject In 1937 and 1939 Wagener and Keith (24 25) published an extensive review of the literature on hypertension and hypertensive eye ground changes and proposed a system of classification (11 24 25) for hypertensive disease based on ophthalmoscopic findings The classification was shown to have predictive properties as to mortality It has been widely adopted and still seems to be the most commonly used classification But it also has been criticized from several points of view The patient series on which the classification was based was highly selected in respect of severity of hypertension 75% of the patients being classified as groups 3 and 4 The prognostic value of groups 1 and 2 containing only 10 and 26 patients respectively has therefore been considered unreliable (1 2) The distinction between groups 1 and 2 is unclear (3) The classification has been regarded as stereotype the same findings meaning completely different things in young persons and elderly individuals (15) The heterogeneity of the groups caused by grouping the features and the subsequent difficulties in reproduction and comparison of the results have also been pointed out (1)

In 1952 Scheie (19) advocated a classification in which hypertensive features such as attenuating arterioles focal narrowings haemorrhages exudates and oedema and arteriolosclerotic features such as broadened reflex crossing phenomena and tortuosity of vessels were classified separately on a 4-point scale He regarded hypertensive changes as a measure of disease severity and arteriolosclerotic changes as a measure of disease duration No data seem to have been put forward to support this hypothesis Most authors agree that hypertensive changes are related to blood pressure (BP) but the significance of arteriolosclerotic changes is

Address for reprints K Svardsudd Medical Department I Sahlgren s Hospital S-413 45 Gothenburg Sweden

still controversial (1 2 4 9 11 12 16, 19 20 23 24 25 26)

Since Wagener and Keith s original publication (24) few evaluations of the prognostic significance of hypertensive eye ground changes have been carried out In only one study (3) has the relationship of prognosis to eye ground changes been analyzed with the importance of BP taken into account In that study the eye ground changes were of importance for mortality However the Wagener and Keith classification was used and no analysis was performed for separate eye ground features

Knowledge of hypertensive eye ground changes in the general population is still fragmentary All studies but two (1 14) have been performed on selected groups of patients We still do not know how well the separate eye ground features and combinations of features correlate to BP in the general population Do they occur at all BP levels? If so are they indicative of a future BP rise? In other words which comes first eye ground changes or hypertension? If hypertension comes first what feature or combination of features best discriminates BP levels? If eye ground changes are a measure of the severity and duration of the hypertensive disease patients with changes should have a poorer prognosis than those of the same age who have no changes but the same BP

The present study—part of a long term investigation of the development of ischaemic heart disease other diseases in a random population sample the same age and sex—is an attempt to answer questions

STUDY POPULATION

All inhabitants of Sweden have a national registration number that includes their date of birth and other vital statistics Names addresses and registration numbers are registered with the official county census bureau and were accessible before the sample was drawn for the present study The study population was recruited from men living in Gothenburg Sweden born in 1913 and still alive at the age 50 years (1963) All men meeting these criteria who were born on a date divisible by three (the 3rd 6th 9th day and so on of each month) comprised the study sample A total of 973 men met these criteria Of these 855 (88%) agreed to be examined in 1963 at Sahlgren s Hospital Gothenburg The base population participants (22) and non participants (21) have been described previously The 855 participants in 1963 were invited to attend a re examination in 1967 and 792 men participated A third examination took place in 1973 when 703 of the 792 men were re-examined

METHODS

Blood pressure was measured in the right arm as casual BP in the seated position after 5 min rest using a mercury sphygmomanometer with a cuff size of 12×23 cm Only one reading was made One observer made all measurements except for 40% of the registrations in 1973 in which two additional observers were involved The examinations in 1963 and 1973 were made in the morning (7–8 a m) and in 1967 in the afternoon (2–3 p m) For this study the systolic and diastolic phase 4 BPs are used

The eye fundi were examined under complete mydriasis Both direct and indirect ophthalmoscopy were used and a fundus photograph was taken The two examinations in 1963 and 1967 were performed by the same ophthalmologist (E A) who was unaware of the participants history and BP The presence or absence of attenuation of the arterioles focal narrowing broadened light reflex crossing phenomena haemorrhages exudates and oedema were noted Presence of the former four variables was defined as slightly" or markedly and the coding used was 1=not present 2=present slightly 3=present markedly (22) Since the number of individuals with code 3 for focal narrowing and crossing phenomena was less than 20 code 3 has been amalgamated with code 2 for these variables Data are missing for one or more of the eye ground variables in 5 men in 1963 and in 25 men in 1967 mainly because of technical difficulties The code values are analyzed numerically and statistics are given in code values

Data on smoking habits were obtained in 1963 For stastical analysis smoking was graded as never smoked=1 stopped smoking=2 smoking 1–14 cigarettes/day=3 smoking 15–24 cigarettes/day=4 smoking 25 or more cigarettes/day=5 Those who smoked cigars cigarillos or a pipe were classified according to their tobacco consumption 1 g of tobacco equalling 1 cigarette The code values are used as numerical values to evaluate heaviness of smoking in some of the analyses

The fasting level of serum cholesterol in 1963 was determined at the same laboratory for all men and regular checks were performed to determine the accuracy of the method (22)

Mortality data for all the 973 males in the original sample have been followed continuously Mortality observations up to 1975 (12½ years) are used for this study Death certificates were available on all deceased subjects The autopsy rate was 87% among the 855 men followed in this study

Morbidity data for stroke and myocardial infarction also up to 1975 have been obtained through case histories and from Nov 1968 onwards also through a myocardial infarction register (7) and a stroke register (8) These registers covering the Gothenburg area include more than 94% of all incidences Special arrangements have been made for individuals leaving the area Criteria for the diagnosis of stroke were hospitalization with the clinical diagnosis of stroke or fresh cerebral thrombosis or haemorrhage at autopsy Subarachnoidal or subdural haemorrhages were not included The criteria for diagnosis of myocardial infarction were hospitalization with the clinical diagnosis of myocardial infarction or fresh coronary heart disease at autopsy The clinical

Table I *Frequency of ophthalmoscopic findings in 855 men examined in 1963 (aged 50) and 792 men examined in 1967 (aged 54)*

The percentages do not add up to 100 since the signs are not mutually exclusive (more than one sign may occur in an individual)

	1963		1967	
	n	%	*n*	%
Attenuating arterioles	131	15 4	216	27 2***
Focal narrowing	51	6 0	60	7 6
Crossing phenomena	76	8 9	77	9 7
Broadened reflex	312	36 7	264	33 4
Haemorrhages	3	0 4	7	0 9
Exudates	0		1	0 1
Papillary oedema	0		0	
No changes	507	59 6	409	53 3

*** $p<0\ 001$

criteria for myocardial infarction were those adopted by the Swedish Society of Cardiology central chest pain shock or syncope suggesting a myocardial infarction together with a typical transaminase spectrum and/or appearance of a pathological Q wave or localized ST variations in the ECG Criteria for coronary heart disease at autopsy were a fresh myocardial scar or in the absence of any macroscopic scar a total or almost total occlusion of a coronary artery together with a medical history suggesting myocardial infarction

For the few individuals with multipe end points for example both survival of first myocardial infarction and subsequent death from other causes the end point occurring first has been used An exception is stroke which because of the small numbers has been used as the end point when present

Statistical methods

Differences in mean values between groups have been tested using the two sample *t* test To evaluate the prognostic importance of the eye ground variables when BP smoking and serum cholesterol are taken into account a multivariate analysis has been performed The analysis consisted of multiple logistic regressions one for each end point together with the eye ground variables smoking cholesterol and systolic BP The method has been described in detail earlier (27) Values of $p<0\ 05$ were regarded as statistically significant using two-tailed tests

RESULTS

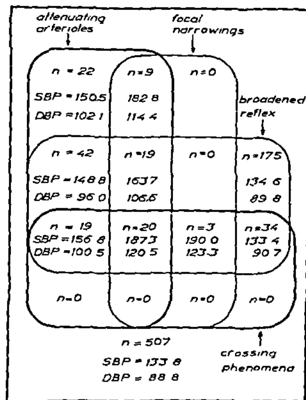
The frequency of hypertensive eye ground abnormalities in the study population examined at 50 and 54 years of age (in 1963 and 1967 respectively) is shown in Table I The dominant feature was broadened light reflex of arterioles observed in approximately one third of the sample Attenuating arterioles occurred in 15–27% and focal narrowing and crossing phenomena in less than 10% Haemorrhages exudates and oedema of the papilla were infrequent Only attenuating arterioles have changed significantly ($p<0\ 001$) in frequency during the four years the frequency having almost doubled The result was the same when individuals who were treated for hypertension were excluded Since haemorrhages exudates and papillary

Table II *Mean systolic and diastolic blood pressure in relation to presence (+) or absence (−) of ophthalmoscopic findings in 1963*

Statistical significance applies to differences in mean values between + and − for each finding

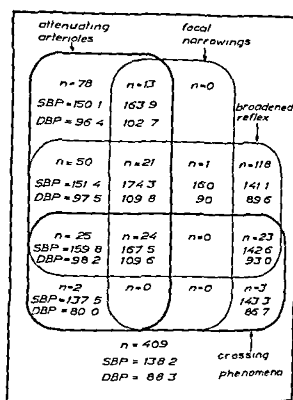
		Systolic BP		Diastolic BP	
	n	X	S D	X	S D
Attenuating arterioles					
+	131	160 6	26 85	104 2	15 28
−	720	134 2	16 74 *	89 2	11 33 *
Focal narrowing					
+	51	177 8	27 74	114 4	15 83
−	801	135 7	17 65 *	90 1	11 57 *
Crossing phenomena					
+	76	155 7	32 26	102 3	17 43
−	776	136 6	18 65	90 5	12 21*
Broadened reflex					
+	312	143 4	24 38	94 7	14 44
−	539	135 3	18 01	89 8	12 07 *

$p<0\ 001$



1963

Fig 1 Grouping according to presence of eye ground findings and combinations of findings at the examination in 1963 *SBP* = mean systolic blood pressure *DBP* = mean diastolic blood pressure



1967

Fig 2 Grouping according to presence of eye ground findings and combinations of findings at the examination in 1967 Abbreviations as in Fig 1

were so few in this study they will not be considered further in this paper

The relation between the other eye ground variables and BP is shown in Table II They are all significantly related to systolic and diastolic BP To see whether these relations are homogeneous or if there are subgroups with differing relations to BP the sample was subdivided into groups according to the presence of the eye ground variables or combinations of them at the examination in 1963 (Fig 1) Five men were not classifiable because of missing data on one or more of the eye ground variables 507 had no eye ground changes 209 had crossing phenomena and/or broadened reflex but no other signs There is no significant difference in mean BP between these two groups The groups with attenuating arterioles but no focal narrowing (83 men) had a significantly higher mean systolic and diastolic BP than the group with no changes ($p<0.001$) No significant differences were seen among the subgroups with attenuating arterioles All men with focal narrowing but three (51 men altogether) also had attenuating arterioles BP was significantly higher in these groups than in the groups with attenuating arterioles but no focal narrowing ($p<0.001$) No significant differences were found among the subgroups with focal narrowing

In Fig 2 the sample is subdivided in the same way for the examination in 1967 The main results are the same as for 1963 Attenuating arterioles in combination with focal narrowing were related most strongly to BP followed by attenuating arterioles with no focal narrowing Isolated crossing phenomena and/or broadened reflex did not seem to be related to BP

Fig 3 illustrates the discriminative power of the eye ground changes for BP Attenuating arterioles without focal narrowing discriminated poorly Focal narrowing discriminated better but only 58% of individuals with systolic BP ≥190 mmHg were

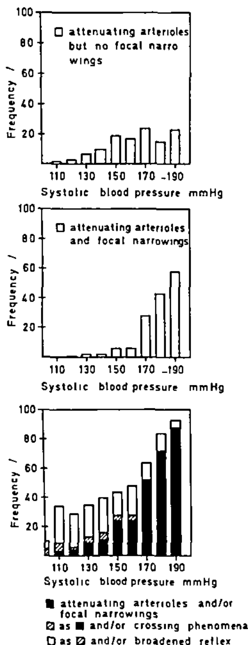


Fig 3 Frequency of men with ophthalmoscopic findings in different systolic blood pressure groups in 1963

detected Attenuating arterioles and/or focal narrowings had the best discriminative capacity and detected 88% of individuals with systolic pressure ≥190 mmHg The addition of crossing phenomena and broadened reflex lessened the discriminative power Comparable results were obtained for diastolic BP

From Figure 3 it is evident that there are individuals with BPs usually not considered indicative of hypertension who have eye ground changes Are these changes indicative of a future BP rise? Table III shows the systolic and diastolic pressures 4 and 10 years after the initial examination in those who had and who did not have attenuating arterioles and/or focal narrowing at the first examination There were no significant differences in BP development between these two groups

The sample was grouped into persons deceased or alive at the end of 1975 regardless of other end points The mortality rates in the groups presented in Fig 1 are given in Fig 4 The rates were essentially the same in the group with no changes and the groups with isolated crossing phenomena and/or broadened reflex Compared with the group with no changes the groups with attenuating arterioles but no focal narrowing had a somewhat but not significantly higher rate The groups with focal narrowings had a significantly higher rate than the groups with no changes ($p<0.001$) and the groups with attenuating arterioles but no focal narrowing ($p<0.05$) Two of the five unclassifiable men died during the 12½ year follow up

The differences in mortality rate could however be explained by differences in BP A multivariate analysis was carried out in order to see whether the eye ground changes influence mortality when BP is taken into consideration i e if men with eye ground changes have a poorer prognosis than men with the same BP but no eye ground changes Since cardiovascular mortality is the dominant cause of death in this study the other two main risk factors for cardiovascular mortality serum cholesterol and smoking should be considered as confounding variables if they are also related to the eye ground variables In this study there was an insignificant trend towards a positive relation of smoking and cholesterol to all eye ground variables except for cholesterol to crossing phenomena for which the trend was significant ($p<0.05$) These relations are thus weak but not negligible To be on the safe side cholesterol and smoking were included in the analysis As systolic and diastolic BP are highly correlated only the systolic pressure was used A multiple logistic regression analysis was performed with the eye ground variables smoking BP and cholesterol entered Focal narrowing and crossing phenomena significantly contributed to mortality regardless of cause ($p<0.05$ and $p<0.01$ respec

Table III *Blood pressure in 1967 and 1973 in those who had and who did not have attenuating arteriole and/or focal narrowing in 1963*

The population is stratified according to systolic BP in 1963
SBP=systolic blood pressure, DBP=diastolic blood pressure AA/FN=attenuating arterioles and/or focal narrowing

SBP in 1963 (mmHg)	AA/FN in 1963			No AA/FN in 1963			AA/FN in 1963			No AA/FN in 1963		
	n	SBP in 1967	DBP in 1967	*n*	SBP in 1967	DBP in 1967	*n*	SBP in 1973	DBP in 1973	*n*	SBP in 1973	DBP in 1973
–145	40	145 1	93 9	545	136 5	88 2	34	145 7	91 2	499	139 4	88 8
150–155	22	155 5	98 6	69	157 5	96 8	19	159 7	98 6	64	166 0	97 9
160–165	11	157 3	100 9	37	164 7	99 1	11	161 6	98 0	35	163 1	97 9
170–175	13	178 1	107 3	12	170 0	105 0	12	170 2	100 2	10	168 6	99 2
180–185	10	160 0	109 0	4	165 0	102 5	9	171 8	108 4	3	174 0	102 0
190–	22	176 6	109 8	4	175 0	103 8	19	180 3	104 7	2	181 0	113 0
Total	118			671			104			613		

tively) even when the influence of smoking BP and cholesterol was taken into consideration

To see if this results is linked to any specific disease or group of diseases the mortality end point was subdivided into deaths due to myocardial infarction other coronary heart disease stroke malignancy and all other causes Individuals surviving strokes were included in the stroke group and persons surviving myocardial infarction were included as a separate group Table IV shows the relationship between the end points and the eye ound variables BP smoking and cholesterol are those who have not reached any of other end points The table gives mean code and mean values for each variable in each end point group For myocardial infarction there was a tendency towards increasing mean values for most of the variables from normal to surviving myocardial infarction to fatal myocardial infarction Focal narrowing crossing phenomena smoking and cholesterol were all significantly associated with myocardial infarction Other fatal coronary heart disease was significantly correlated to attenuating arterioles crossing phenomena and BP A similar analysis for stroke morbidity revealed significant relationships between stroke and all eye ground variables and BP For cancer mortality BP and focal narrowing were of significant importance and for death due to other causes crossing phenomena broadened reflex and smoking were of importance

Table V shows the result of a multivariate logistic regression analysis of the relations of the eye ground variables to the end points when smoking BP and cholesterol were corrected for The surviving and fatal myocardial infarction end points were pooled as were the fatal myocardial infarction and

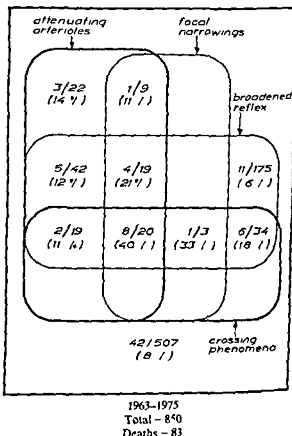


Fig 4 Mortality rate during 1963–75 for the groups presented in Fig 1 Number of deaths in relation to number at risk Five men were not classifiable because of missing data on one or more of the eye ground variables Two of these men died during the period

Table IV *Mean code values for the eye ground variables and smoking and mean values for systolic blood pressure and cholesterol in groups according to end point*

The mortality end points do not add up to 85 because of competing end points

	Normal (n=718)	Myocardial infarction Surviving (n=40)	Myocardial infarction Fatal (n=14)	Other fatal coronary heart disease (n=8)	Stroke (n=16)	Fatal malignancy (n=20)	Other causes of death (n=29)
Attenuating arterioles[b]	1 22	1 26	1 47	1 63*	1 59**	1 45	1 36
Focal narrowing[c]	1 05	1 02	1 19*	1 13	1 35***	1 20**	1 11
Crossing phenomena	1 07	1 09	1 25**	1 38**	1 35***	1 05	1 21**
Broadened reflex[b]	1 42	1 37	1 63	1 75	1 82*	1 20	1 76*
Smoking[d]	2 48	3 28 **	3 38**	2 75	2 94	2 75	2 93*
Systolic blood pressure (mmHg)	136 9	142 5	146 6	153 8*	158 8***	145 8*	139 3
Cholesterol (mg/100 ml)	244 6	260 3	276 9**	261 0	264 5	246 7	254 6

[a] No myocardial infarction or stroke and alive at the end of 1975
[b] Code 1=no 2=yes slightly 3=yes markedly
[c] Code 1=no 2=yes
[d] Code 1=non smoker 2 ex smoker 3=smoking 1–14 g/day 4=smoking 15–24 g/day 5=smoking ≥25 g/day
Significant deviations from the normal group $*p \leq 0$ 05 $**p \leq 0$ 01 $***p \leq 0$ 001

other fatal coronary heart disease end points to get a sufficient number of individuals in each end point No single eye ground variable seemed to be of importance for myocardial infarction Focal narrowings were of importance for stroke morbidity and cancer mortality Crossing phenomena were important for fatal coronary heart disease stroke morbidity and mortality from causes other than coronary heart disease stroke or malignancy Broadened reflex had importance for stroke when smoking BP and cholesterol were kept constant but was not significant when crossing phenomena were taken into consideration

Multiple linear stepwise regression analyses were performed to compare the predictive power of the significant eye ground variables in Table V with the predictive power of smoking *BP* and cholesterol *In* each end point group smoking BP cholesterol and the significant eye ground variables were included in the analysis In the first step the variable with the highest predictive power was included In step two the variable among those still not included with the highest predictive power was included and so on until all variables had been entered The significant eye ground variables in Table V were consistently the first variables included and are thus the best single predictors of the variables analyzed for these end points

DISCUSSION

Hypertensive eye ground changes have long been considered to be stable easily detectable and classifiable signs of hypertensive disease The stability is one of the reasons for ophthalmoscopic examination in hypertension since the changes are thought to reflect the *BP* level and severity of the disease better than casual BP measurements The presumed ease of detection and classification is reflected in

Table V *P values (indicated if $p \leq 0$ 05) for the relations of eye ground variables to mortality and morbidity after correcting for smoking blood pressure and cholesterol in multiple logistic regression analyses (all relations are positive)*

	Myocardial infarction (n=54)	Fatal coronary heart disease (n=22)	Stroke (n=16)	Fatal malignancy (n=20)	Other causes of death (n=29)
Attenuating arterioles					
Focal narrowing			0 5	0 05	
Crossing phenomena		0 03	0 02		0 02
Broadened reflex			0 05		

the Wagener and Keith classification where the degree of change is the only factor distinguishing groups 1 and 2 However Kagan et al (10) in a study of the reproducibility of eye ground findings on the basis of only two degrees present or absent found large interobserver (20–42%) as well as intra observer (10–33%) variation in spite of using fundus photographs and well trained observers This variation was largest for the four variables used in this study but was also pronounced (8–17%) for haemorrhage exudate and papilloedema Co-training and standardization did not increase the precision Like any other variable hypertensive eye ground changes are thus subject to measurement and classification errors The errors are of importance when the variables are used to classify individuals for example in a prognostic analysis They tend to dilute or conceal existing relationships The risk of their causing false relationships is small (5 18) For description of groups the errors are less important Since the observer was unaware of the men s histories and BP the classification errors may be assumed to be randomly distributed over the groups and thus cause no serious bias

Most authors agree that attenuating arterioles and focal narrowing are related to the BP level (1 2 3, 4 9 11 19 20 24 25 26) These changes are generally interpreted as vascular damage due to the hypertensive process attenuation being an early sign and focal narrowing a more advanced one (20 24 25) Some authors have also found a correlation to age (2 9 23) Even before Wagener and Keith broadened reflex and crossing phenomena were considered to be caused by arteriosclerosis crossing phenomena being the more pronounced arteriosclerotic feature (24) Subsequent morphological studies have supported this hypothesis (11 13 24 25 26) Most authors have found a correlation to both BP and age (2 4 9 12 23 26) the disagreement has concerned whether BP or age is the main determinant Bechgaard et al (2) found no correlation between broadened reflex and age and van Buchem et al (4) found no correlation to BP Whether they investigated isolated broadened reflex or reflex regardless of other signs is unclear Aurell and Tibblin (1) found no correlation between isolated broadened reflex and/or crossing phenomena and diastolic BP In the present study all the four eye ground variables were independently related to BP but subgrouping showed that isolated broadened reflex and/or crossing phenomena were not If eye ground changes are used for diagnostic purpose in hypertensive disease attenuating arterioles and focal narrowing should be the variables to be used Attenuation has the best discriminating power of all the variables In terms of sensitivity (ability to detect hypertensives) and specificity (ability to exclude normotensives) it has a high sensitivity and a rather high specificity Focal narrowing adds further information It has a rather low sensitivity but a high specificity The presence of attenuating arterioles in a patient makes it probable that the BP is raised the presence of focal narrowing makes it highly probable Crossing phenomena and broadened reflex do not add anything further in this respect and should therefore be disregarded for this purpose

Since BP is known to influence the mortality regardless of cause and morbidity in stroke and myocardial infarction one would expect hypertensive eye ground changes also to be related to these events Wagener and Keith showed this to be true for overall mortality But the point of interest is whether they are of importance when BP is taken into account Breslin et al (3) showed that the modified Wagener and Keith classification was of independent prognostic importance for mortality No previous investigation of the importance of the separate eye ground variables has been performed In this study focal narrowing was of importance for morbidity in stroke and mortality in cancer and as a reflection of that was also correlated to mortality regardless of cause Since it most probably represents the arteriolar damage caused by the hypertensive process it was expected that such damage is of importance for development of stroke The correlation to cancer mortality was unexpected and is harder to understand It does not seem to be just a reflection of the association between BP and cancer found in this and other studies (6) It may indicate that the BP–cancer relationship is time-dependent being most pronounced in long standing hypertension in the same way as the reported relationship between reserpine and breast carcinoma (17) has sometimes been interpreted as a relationship between long standing hypertension and cancer Crossing phenomena were of importance for stroke as expected They were also of importance for other fatal coronary heart disease dominated by sudden deaths where advanced coronary arteriosclerosis—but no definite signs of myocardial infarction—could be found at the postmortem ex

amination This fits in well with the concept of crossing phenomena as a measure of the arteriosclerotic process They were also of importance for death from other causes though not associated with BP indicating that individuals with more advanced arteriosclerosis have a higher mortality risk also in non cardiovascular diseases than individuals with less advanced arteriosclerosis

These four eye ground variables are thus of differing and supplementary importance Any grouping of them means loss of information If for example Wagener and Keith s classification had been used group 1 would probably have included isolated broadened reflex and/or attenuating arterioles Group 2 would have included crossing phenomena and/or attenuating arterioles and/or *focal narrowing and/or haemorrhages The groups* become highly heterogeneous and the results correspondingly hard to reproduce When this classification was introduced it brought order into the chaotic situation in the field of hypertension But to-day when groups 3 and 4 are small and almost all hypertensives belong to groups 1 and 2 this classification obscures more than it clarifies The best system of classification to-day is that based on the separate eye ground features themselves classified on a 2 point scale—present or absent

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Antihypertensive Treatment with Pindolol in One or Two Doses

A Comparative Trial

Finn Gyntelberg Knud Rasmussen and Bent Harvald

From Department of Internal Medicine C Bispebjerg Hospital Copenhagen Department of Internal Medicine C Diakonissestiftelsen Frederiksberg and the Department of Internal Medicine Odense University Hospital Odense Denmark

ABSTRACT A comparative trial was carried out in 21 patients on the antihypertensive effect of pindolol given once or twice a day A change-over design was employed with two treatment schedules and four treatment periods of six weeks each No statistically significant difference was found in BP lowering effect between one and two daily doses It is unlikely that a switch from pindolol twice daily to the same amount once daily will increase the BP by more than at most 1 0 mmHg systolic and 2 2 mmHg diastolic, or decrease it by more than 2 7 and 1 0 mmHg, respectively It is concluded that pindolol given once daily can be recommended

In a few non comparative studies pindolol (Visken®) was found to be efficient as a BP lowering drug given once daily (3 5 6 7) Since non compliance to antihypertensive treatment is a major clinical problem (1 9) and once daily medication regime is easier to adhere to than the usual two or three times daily (9) we found it worthwhile to compare two medication regimes of pindolol a once or twice daily medication

The questions we wanted to answer were (*a*) Would the BP values differ significantly between two periods in which pindolol was given once or twice daily? (*b*) Would side effects be different? (*c*) Which dosage scheme would the patients prefer?

PATIENTS AND METHODS

Twenty four consecutive patients with high BP attending three hypertension clinics in Copenhagen Frederiksberg and Odense were included in the trial The following criteria for inclusion were applied Hypertension according to WHO stages I or II Diastolic BP≤100 mmHg on a pindolol dose not exceeding 20 mg daily for more than 4 weeks

Excluded were patients who had previously been treated with Visken in one daily dose patients who needed more than 20 mg pindolol a day and patients who needed other antihypertensive drugs apart from thiazides or chlorthalidone Usual contraindications to β-blocking therapy were considered Three patients had to be excluded for the following reasons one started a diuretic during the trial one started another drug the dosage of which was altered during the trial and one moved temporarily to another city during the trial

Two women and 19 men median age 51 5 years (range 34–67) completed the trial Their mean height was 174 cm (range 156-190) and mean weight 80 3 kg (range 49–96) The duration of diagnosed hypertension ranged from 1 to 10 years (median 5 0)

Fourteen patients had hypertension classified as WHO stage I and seven as WHO stage II Four patients had received no previous treatment four had diuretics alone six a β blocking agent alone and seven a β blocking agent plus a diuretic before inclusion in the trial The mean dose of pindolol given was 10 2 mg and five patients reveived a diuretic in addition

The trial was carried out as a fixed sample size trial A change-over design with two treatment schedules and four 6 weeks treatment periods was employed Schedule 1 was pindolol once daily schedule 2 pindolol twice daily The total daily dosage of pindolol was the same with the two schedules and equal to the daily dosage prior to the trial Patients were allocated at random to one of the eight possible treatment sequences (1 2 2-1 1 2 2-2 1 2 1 etc) BPs were recorded by trained nurses who were blindfolded as to treatment schedule No other blinding was however employed

The trial started in Dec 1975 and was planned to stop when all patients had been through the four treatment periods Each patient was treated during four periods of at least six weeks duration BPs were measured every second week in the morning before the first pindolol dosage Each time three measurements were made to the even cipher with the patient same sphygmomanometer and standar all measurements in one and

Table 1 *Blood pressure (mmHg) in 21 patients during treatment with pindolol in one (schedule 1) and two (schedule 2) daily doses (mean±S D range in parentheses)*

			Difference	
BP	Schedule 1	Schedule 2	1–2	95% Confidence limits
Systolic	147 1±14 2 (123 7–179 5)	148±13 7 (124 8–181 7)	−0 84±4 03	(−2 67– +0 99)
Diastolic	97 8±5 5 (87 7–111 6)	97 2±5 2 (85 9–110 9)	0 58±3 56	(−1 04– +2 20)

Statistical analysis

The principal statistical method was parametric analysis of variance The assumptions of homoscedasticity were met but the assumptions of normal distributions not always Accordingly BPs were analyzed by the Friedman test also On no occasion did conclusions differ

RESULTS

No statistically significant differences were found between patients who started with Visken once or twice daily ($p>0$ 10) Neither were there any significant systemic within patient variations due to period effects residual treatment effects etc (all values >0 10) Differences between BPs on the treatment schedules were numerically small statistically insignificant (all p values >0 10) Findings are summarized in Table 1 Confidence limits for the difference between BPs on the two schedules indicate that it is unlikely that a switch from Visken twice daily to the same amount in a single dose will increase systolic BP by more than at the most 1 0 mmHg and diastolic by more than at most 2 2 mmHg It is on the other hand also unlikely that BP will decrease by more than 2 7 mmHg systolic and 1 0 mmHg diastolic at the most These differences are so small that the treatments must be regarded as having an equal BP lowering effect

Side effects

Thirteen patients (62%) had no side-effects The others had brief unsystematic minor symptoms with the same frequency on both treatments

Patient preference

Of the 21 patients only two preferred the twice daily treatment

DISCUSSION

The conclusion that can be drawn from the data presented here is very clear Pindolol given in a *single daily dose can be recommended* as the BP lowering effect was equal to that of a twice daily dose with no extra side-effects and a predominance in patient preference Thus this comparative trial supports the data from previous non-comparative trials on pindolol in one daily dose While this study was in progress Jacobsson (10) published data on another comparative trial on pindolol which gave results similar to ours In addition Jacobsson used exercise tests but he did not randomize his patients to one or the other treatment from the start of his trial All his patients started on a two dose regimen Also atenolol has been shown to be effective when given once daily (4) and so has propranolol (11) It is not unlikely that several other β blockers in the *same way could be given in a single daily dose* The clinical effect of β blockers may not reflect their kinetic characteristics Thus plasma half life of pindolol is between four and six hours (8) Considering the practical importance of giving β blockers in one daily dose for patient compliance more comparative studies using other β-blockers should be carried out

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Oesophageal Function and Coronary Angiogram in Patients with Disabling Chest Pain

Olof Svensson Goran Stenport Lita Tibbling and Bengt Wranne

From the Departments of Medicine (Division of Cardiology) Radiology Otorhinolaryngology and Clinical Physiology University Hospital Linkoping Sweden

ABSTRACT Sixty four patients with a history of disabling chest pain belonging to groups III or IV classified according to the NYHA criteria were examined with oesophageal function tests, coronary angiography and bicycle ergometry and also answered a symptom questionnaire At the exercise test, 52 had effort angina 45 (89%) of them had a pathological coronary angiogram and 22 (42%) had signs of oesophageal dysfunction (OD) OD as the single possible etiological factor for typical effort angina therefore seemed unlikely Chest pain was absent or atypical at the exercise test in 12 patients, 11 (92%) of whom had signs of OD This incidence is significantly higher ($p<0\ 01$) than that found in the patients with effort related chest pain Five (42%) of the 12 patients with atypical chest pain at the exercise test had a pathological coronary angiogram, an incidence which is significantly lower ($p<0\ 001$) than that found in the group with effort related chest pain In patients with a history of disabling chest pain but with atypical chest pain in connection with the exercise test, OD was more frequent than coronary disease and therefore more likely to have caused the symptoms

Key words Oesophageal function ischaemic heart disease effort angina coronary angiography exercise ECG
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Bennett and Atkinson (3) pointed out that oesophageal dysfunction (OD) is common in patients with chest pain referred to the emergency room and Kramer and Hollander (9) showed that balloon distension of the oesophagus may cause pain which mimics that in myocardial infarction It seems clear from these reports and others (4 5 6 8 10 12 13) that pain of oesophageal origin is often similar to cardiac pain with regard to localization and radiation The reports however do not give convincing evidence that effort angina can be caused by OD

Tibbling and Wranne (17) found a high incidence of OD in patients referred to an exercise electrocardiography (ECG) because of a history of chest pain The incidence was highest in the group with a negative effort angina questionnaire (14) but a high incidence of OD was found even in the group with normal exercise ECG and positive Rose questionnaire This raised the question as to whether OD was the cause of effort angina in these patients The diagnosis of ischaemic heart disease (IHD) was for the purpose of that study based on ECG at rest and exercise and not on coronary angiogram The incidence of IHD may therefore have been underestimated

The aim of the present paper is therefore to study the incidence of OD in patients with chest pain in whom the investigation also included coronary angiogram and to find out if and to what extent OD may be the cause of effort angina

PATIENTS AND METHODS

The study comprised 54 patients (mean age 50 years) with a history of typical effort angina and 10 patients (mean age 51) with a history of atypical angina i e attacks of chest pain not precipitated by effort All patients were classified as belonging to groups *III* or *IV* according to the functional criteria set by the NYHA (11) Vigorous medical treatment including high doses of β adrenergic blocking drugs had failed to give acceptable improvement The patients were therefore subjected to further investigation which included chest X ray resting and exercise ECG oesophageal manometry including an acid perfusion test coronary angiography and in most patients also a right heart catheterization and a left ventricular angiography They were also asked to fill in a questionnaire aiming at detecting complaints of possible oesophageal origin and to register their smoking habits

The coronary angiography was carried out by the Judkins technique (7) with different catheters for the left and

Abbreviations OD=oesophageal dysfunction ECG=electrocardiography IHD=ischaemic heart disease CHD=coronary heart disease

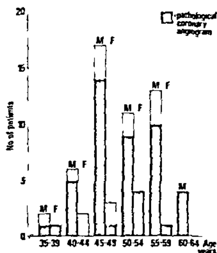


Fig 1 Result of coronary angiography by age and sex

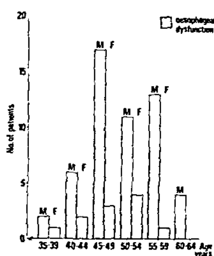


Fig 3 Oesophageal dysfunction by age and sex

right coronary arteries The angiograms were viewed without knowledge of clinical status and were considered pathological if one or more of the central coronary arteries had stenosis of more than 70% of the estimated luminal area At the catheterization pulmonary capillary venous and/or left ventricular diastolic pressure was determined at rest and during recumbent near maximal bicycle ergometry The values reported here are obtained at near maximal recumbent bicycle work A value of more than 15 mmHg was regarded as abnormal

A 12 lead ECG was registered at rest and evaluated according to the Minnesota Code (15) Sitting exercise was performed on an electrically braked bicycle ergometer with stepwise increase of loads every 6th min using chest pain or fatigue as end-point The patients were asked to abstain from smoking eating major effort or nitro intake for 4 hours prior to the test The ECG was registered recumbent before during 10 min after the exercise (extremity + V leads) as well as continuously durr exercise with the forehead as reference (CH_{1-4})

The ECG changes during exercise were classified ac cording to Areskog et al (2) ST changes during wor being classified from 0 to 3 points Changes within 0 1 m are scaled as 0 and a junctional ST depression of 0 3 m or more with form change as 3 T wave changes afte exercise are also scaled from 0 to 3 0 denoting mino changes after work with successive restitution of the wave amplitude and 3 a transient change in the T wav amplitude to less than −0 2 mV within 2–4 min after work without parallelity to decrease in heart rate The sum o the S–T and T wave scaling was calculated A typica ECG reaction for coronary insufficiency was defined a 3–6 points a borderline reaction as 2 points and a negativ reaction as 0–1 point Of 6 patients receiving digitali none had a pathological ECG reaction

Chest pain in connection with the exercise test wa classified by a trained physician from 0 to 3 consider character site and time course 0 is no pain 1 atypica pain 2 almost typical pain and 3 is typical anginal pain In the further presentation groups 0–1 will be referred to as atypical and groups 2–3 as typical anginal chest pain

Oesophageal function was evaluated by manome ry and

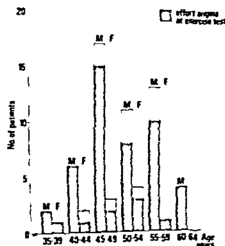


Fig 2 Chest pain at exercise test by age and sex

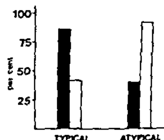


Fig 4 Incidence of pathological coronary angiogram (■) and oesophageal dysfunction (□) in 52 patients with typical and 12 with atypical anginal chest pain at exercise test.

Table I *Patients with normal coronary angiogram*

Sex	Age (y)	History of angina pectoris	Chest pain at exercise test[a]	Exercise ECG	OD	LV_{ED} PCW (mmHg)[d]	Note
♂	46	−	3	0	−		
♂	47	+	3	0	+	30	Atrial fibrillation
♀	48	−	3	3	−	18	
♂	58	+	3	1	−	24	
♂	59	−	3	PI	−	13	
♂	37	+	2	0	+		Earlier pericarditis
♀	41	−	2	0	−	24	
♀	42	−	1	0	+		
♂	44	−	1	0	−	20	Atrial fibrillation
♂*	50	+	1	0	+	14	
♂	53	+	1	0	+	15	Branch stenosis
♂	57	−	1	2	+	18	Branch stenosis
♀	46	+	0	PI	+	30	
♂	49	−	0	0	+	14	Atrial fibrillation + cardiomegaly

No cardiovascular abnormalities at the time of investigation

[a] Graded 0–3 2 and 3 = typical or near typical angina pectoris 1 = atypical angina

Graded 0–6 2 borderline finding 3–6 = pathological (ref 2) PI = ECG signs of old myocardial infarction (ref 15)

[d] Intravascular pressures (LV_{ED} or PCW) measured at near maximal exercise and a pressure of >15 mmHg regarded as abnormal

by the acid perfusion test (1 4 16) The manometry included a tonus test a motility test and an abdominal compression test with registration of oesophageal tonus pressure and pH in the distal part of the oesophagus

The patients were classified as having OD if at least one of the following four criteria were met 1) Positive acid perfusion test 2) Manometrically verified hiatal hernia with a length of 2 cm or more without abdominal compression 3) Dysmotility (i e simultaneous contractions at a length of 10 cm at swallowing in 2 of 10 tested levels in the oesophagus) combined with a lower oesophageal sphincter hypotonia (i e pressure gradient between the lower oesophageal sphincter and the oesophagus at end of expiration of less than 9 mmHg (16)) or combined with oesophageal reflux as diagnosed by a decrease in pH in the distal part of the oesophagus with application of an extra abdominal pressure of 100 mmHg 4) Severe dysmotility (i e simultaneous contractions at a length of 10 cm at dry swallowing in at least 3 of 10 tested levels in the oesophagus) A lower oesophageal sphincter hypotonia or oesophageal reflux as only finding was not classified as OD

Fisher s exact test was used for statistical analysis

RESULTS

Fifty of the 64 patients had a *pathological coronary angiogram* Age and sex distribution is displayed in Fig 1 Forty five of these 50 patients had typical anginal chest pain at the exercise test Five patients had atypical chest pain but 4 of them had ECG signs of an old infarction at rest and the fifth a borderline exercise ECG

Fourteen patients had a *normal coronary angiogram* seven of whom had typical anginal chest pain at the exercise test an incidence which was

Table II *Five patients with pathological coronary angiogram but no or atypical chest pain at exercise test*

Definitions and abbreviations as in Table I

Sex	Age (y)	History of angina pectoris	Chest pain at exercise test	Exercise ECG	OD	LV_{ED} or PCW (mmHg)
♂	48	+	1	PI	+	24
♀	53	+	0	PI	+	30
♂	55	+	0	PI	+	12
♂	56	−	0	PI	+	38
♂	59	−	1	2	+	29

Abdominal pain at exercise test

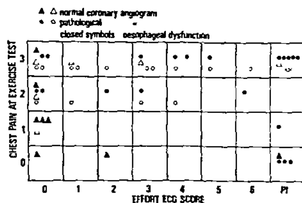


Fig 5 Findings at the coronary angiography and at oesophageal function tests in relation to perceived chest pain and effort ECG Definitions and abbreviations as in Table I

significantly lower than that found in the patients with pathological coronary angiogram ($p<0.001$) Four of these 14 patients had ECG signs of an old infarction or a pathological exercise ECG while 6 had other cardiovascular abnormalities Details regarding these patients are given in Table I Thus 4 patients had no signs of cardiovascular abnormalities at the time of investigation

A total of 33 patients had signs of OD (Fig 2) The incidence of OD in patients with normal coronary angiogram (8/14) did not differ significantly from that found in patients with pathological coronary angiography (25/50)

Fifty two of the 64 patients experienced *typical anginal chest pain* at the exercise test (Fig 3) Of the 12 patients who had *atypical chest pain* at the exercise test 5 had a pathological coronary angiogram an incidence which is significantly lower than that found in the group with typical chest pain (45/52) ($p<0.001$) Eleven of the 12 patients with atypical chest pain and 22 of the 52 with typical chest pain at the exercise test had signs of OD The difference is significant ($p<0.01$) (Fig 4) Details of the patients with atypical chest pain are given in Tables I and II Of the 7 patients with typical effort angina at the exercise test but with normal coronary angiogram only 2 had signs of OD (Table II) The *individual correlations between perceived chest* pain at the exercise test coronary angiogram oesophageal findings and ECG findings at rest and exercise are displayed in Fig 5

When comparing *symptoms* with objective findings the patient group with OD differed significantly ($p<0.001$) concerning symptoms of dyspepsia from that with a normal oesophageal function (Table III) The two groups also differed significantly with respect to precipitation of chest pain by emotional distress or cold environment ($p<0.05$) Sensation of a lump in the throat was more common

ble III *Symptoms in 64 patients given in questionnaire*
D=oesophageal dysfunction PCA=pathological coronary angiogram

	OD		PCA	
	No ($n=31$)	Yes ($n=33$)	No ($n=14$)	Yes ($n=50$)
Do you often have heartburn?	2	14**	3	13
Do you often have acid regurgitations?	1	13* *	4	10
Do you often feel a lump in your throat?	7	11	1	17
Do you often feel surfeited after a meal?	8	14	3	19
Do you get chest pain in connection with exercise?	28	31	13	46
Does the pain disappear if you stop?	20	18	7	31
Does your chest pain improve when lying with your head raised?	5	10	3	12
Does your chest pain get worse at night or when lying down?	7	5	3	9
Do you sometimes get chest pain in a cold environment?	21	30	10	41
Do you get chest pain at emotional distress?	18	28*	9	37
Do you suffer from severe chest pain?	23	26	10	39
Are you a smoker?	15	12	2	25*
Are you an ex smoker?	7	15	5	17
CHD	25	25		
OD			8	25

*$p<0.05$ ***$p<0.001$

in patients with pathological coronary angiogram than in those without ($p<0.05$) The incidence of smokers was higher in the group with pathological coronary angiogram than in that without ($p<0.05$)

DISCUSSION

The complaint in common was incapacitating chest pain The patients were clinically judged as having coronary heart disease (CHD) and the coronary angiography was performed in order to see whether a coronary bypass operation was possible or not The incidence of OD in the group as a whole was significantly higher than in a normal population of comparable age (16) but there was no difference in incidence of OD between the groups with normal or pathological coronary angiogram But when the patients were subgrouped according to chest pain reaction at the exercise test a significantly higher incidence of OD was found in the group with atypical chest pain than in that with typical effort angina ($p<0.01$) We have previously found a high incidence of OD in patients referred to exercise ECG because of history of chest pain but in whom chest pain was not provoked at the test (17) A similar finding in patients with atypical angina was reported by Henderson et al (6) These observations all indicate that OD is common in patients with atypical angina Furthermore the association between symptoms related to the oesophagus and objective findings of OD indicates that if in the evaluation of patients with chest pain specific oesophageal questions are asked as often as specific questions of effort angina the case history already may lead us nearer the true reason for the chest pain The incidence of OD was significantly lower in the patient group with typical effort angina at the exercise test than in the group with atypical angina The opposite was true regarding the incidence of pathological coronary angiogram ($p<0.001$) (Fig 4) OD as the *single* possible etiological factor for chest pain in patients with typical effort angina at an exercise test is therefore unlikely On the other hand OD is fairly common even in this patient group and we have occasionally seen improvement of effort angina after institution of oesophageal therapy in patients with proven CHD

In conclusion our data suggest that in patients with typical effort angina as observed at an exercise test and in whom other causes of ischaemia (e g aortic stenosis) are excluded CHD is the most likely cause of chest pain and OD may or may not add to the symptoms In patients with atypical angina at the exercise test even with a history of cardiac disease OD is more frequent than CHD and therefore more likely to cause the chest symptoms Oesophageal function should be tested in these patients and oesophageal therapy should be instituted and evaluated before routinely subjecting them to coronary angiography All patients with chest pain should also be specifically asked about oesophageal complaints

ACKNOWLEDGEMENTS

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Effect of Nifedipine (Adalat®) on Coronary Haemodynamics in Patients with Coronary Arteriosclerotic Disease

Svein Simonsen and Sigurd Nitter Hauge

From the Laboratory of Cardiology Medical Department B University Hospital Rikshospitalet Oslo Norway

ABSTRACT Effects of the new Ca^{++} antagonist nifedipine (Adalat®) on coronary haemodynamics were studied in 8 patients with documented ischaemic heart disease The continuous infusion thermodilution technique was used to measure cardiac venous blood flow Sublingual application of 10 mg nifedipine caused a significant increase (16%) in myocardial blood flow and a decrease (18%) in coronary arteriolar resistance at rest, but not during a submaximal atrial pacing test There was no change in coronary arteriovenous oxygen difference myocardial oxygen consumption oxygen consumption per unit of heart rate blood pressure index or left ventricular efficiency index The effects on the coronary haemodynamics are discussed in relation to the simultaneous changes in general haemodynamics Systolic aortic pressure was slightly reduced significantly only at rest while peripheral vascular resistance decreased and cardiac output increased also during atrial pacing No change in free fatty acid metabolism was observed It is concluded that nifedipine is a mild coronary vasodilator No effect was observed on myocardial oxygen demand The oxygen cost of left ventricular work was unchanged by the drug both at rest and during the submaximal stress test

Nifedipine (Adalat®) belongs to the so-called Ca^{++} antagonists (4 20) These drugs inhibit the excitation–contraction coupling influence of calcium ions on the cellular membrane The oxygen requirement of the myocardium is highly Ca^{++} sensitive and their effect on the myocardium is supposed to be restricted to the metabolism in which calcium plays a key role Calcium antagonists also have a powerful effect on vascular smooth muscle where at a given pH the tonus depends on the availability of calcium

From a pharmacological point of view an oxygen saving effect of a calcium antagonistic agent would be of clinical importance in the therapy of patients with coronary artery disease Clinical investigations have also demonstrated that nifedipine increases exercise performance in patients with moderately severe angina pectoris and delays the onset of exercise induced chest pain (5 13) This led us to study the effect of nifedipine on coronary and general haemodynamics in patients with ischaemic heart disease (IHD) The main purpose was to study myocardial blood flow and oxygen consumption in relation to the cardiac work performed at rest and during a stress test

PATIENTS

Eight patients with IHD without additional heart disease were investigated Before the study informed consent was obtained from all the participants whose mean age was 49 years (range 36–58) Five patients had more than 50% stenosis on all three major coronary arteries while 3 had significant stenosis limited to the ramus descendens anterior of the left coronary artery Left ventricular ejection fraction was 61% ±3 (S E M) No drugs were given in the last 24 hours before the investigation The patients were examined in the supine position in the over night fasting state after premedication with 0 1 g allypropymal

METHODS

Catheterizations

A special preshaped 7F two thermistor thermodilution catheter (Wilton Webster Lab) with pacing electrodes was positioned in the coronary sinus In six patients the catheter was advanced further into the great cardiac vein but this was not possible in the remaining two Earlier investigations (15) have demonstrated that changes in

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Abbreviations IHD=ischaemic heart disease HR=heart rate BP=aortic pressure LVFP=left ventricular filling pressure $\dot{Q}$=cardiac output LVW=left ventricular minute work LVW/MVO_2=left ventricular efficiency CVF=cardiac venous blood flow MVO_2=myocardial oxygen consumption FFA=free fatty acid(s) FFA = FFA uptake

Table I *Coronary and general haemodynamic variables before and after 10 mg nifedipine sublingually (mean ±S E M)*

	Rest			Submaximal pacing		
	Before	After	Significance of difference	Before	After	Significance of difference
Coronary haemodynamics						
CVF (ml/min)	103±13	119±16	<0 05	136±17	146±17	NS
Coronary arteriovenous oxygen difference (ml/l)	118±6	112±5	NS	118±5	112±6	NS
MVO_2 (ml/min)	12 4±1 9	13 4±1 9	NS	16 0±2 1	16 5±2 2	NS
Coronary arteriolar resistance (mmHg/ml/min)	1 04±0 13	0 85±0 09	<0 05	0 81±0 11	0 72±0 11	NS
MVO_2/HR BP 10^{-4} (ml/mmHg beat)	15 3±3 5	15 4±2 5	NS	11 0±1 8	12 2±1 8	NS
LVW/mVO_2 (gm/ml)	0 81±0 11	0 81±0 09	NS	0 65±0 11	0 67±0 11	NS
FFA arterial concentration (μmol/l)	680±49	636±54	NS	656±42	642±66	NS
FFA_{μ} (μmol/min)	10 1±1 1	9 0±1 8	NS	11 6±2 1	12 7±3 1	NS
General haemodynamics						
HR (beats/min)	67±4	71±2	NS	118±3	118±3	NS
BP systolic (mmHg)	128±5	125±5	<0 05	124±5	117±4	NS
BP mean (mmHg)	96±3	91±3	<0 01	97±3	94±3	<0 05
Pulmonary capillary wedge pressure (mmHg)	6 0±1 2	6 1±1 3	NS	9 4±2 0	9 0±1 4	NS
$\dot{Q}$ (l/min)	5 2±0 3	5 9±0 1	<0 01	5 8±0 1	6 4±0 2	<0 01
Stoke volume (ml)	78±3	84±2	<0 01	49±3	55±3	<0 01
Peripheral vascular resistance (dynes/sec/cm^{-5})	1 568±107	1 314±101	<0 01	1 425±96	1 232±70	<0 01
HR BP index (beats/min mmHg)	8 653±606	8 794±378	NS	14 549±558	13 837±661	NS
LVW (gm/min)	8 8±0 7	9 7±0 5	<0 05	9 0±0 8	9 5±0 7	NS

NS=not significant

venous flow (CVF) from different myocardial re parallel each other A Swan Ganz thermodilution theter was placed in the pulmonary artery and a lyethylene catheter in the thoracic aorta

Measurements

CVF was measured by the continuous infusion ther modilution method (5) in which a 0 9% saline solu tion was infused at a rate of 36 ml/min for 20–30 sec In our laboratory this method has been found reliable with a mean difference between duplicate measurements performed within 1 min of 3 3% for the coronary sinus ostial flow and 3 5% for the great cardiac vein (15) Aortic and pulmonary artery pressures were measured via the indwelling catheters using EMT 35 Elema Schönander Stockholm (ES) transducers and recorded on a Mingograph 800 (ES) ink jet recorder Ten consecutive beats were analysed and the average pressures were used Mean pressures were obtained by electrical integration Cardiac output ($\dot{Q}$) was determined by thermodilution using a cardiac output computer Edwards Laboratories model 9510

Procedure

The patient s angina threshold was tested by atrial pacing using an external pacemaker at a slowly increasing rate until typical anginal chest pain was experienced CVF was measured first at rest and thereafter at a submaximal pacing frequency about 10/min below the rate previously found to produce chest pain Blood samples for determina tion of Hb haematocrit oxygen saturation and free fatty acids (FFA) were taken from the cardiac vein and the aorta immediately before each measurement Intravascu lar pressures were recorded simultaneously with and $\dot{Q}$ immediately after the flow measurements Nifedipine 10 mg was given sublingually and 15 min later the complete procedure was repeated using the same pacing frequency as before

Calculations

CVF was calculated from the formula

$$F_i \ 1\ 19 \left(\frac{T_B - T_I}{T_B - T_M} - 1\right)$$

where T_B T_I and T_M represent the temperature of blood injectate and mixture of blood and injectate respectively F_I is the volume of injectate and 1 19 is a constant derived from the density and specific heat of saline solution and blood (5)

MVO_2=(arterial−cardiac venous oxygen content)

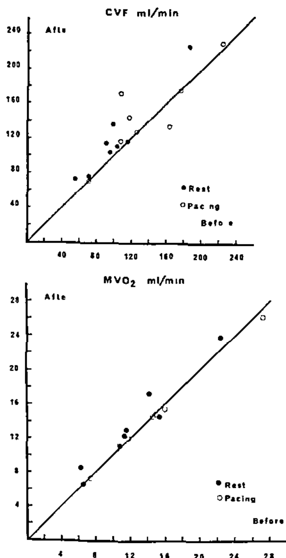


Fig 1 Effect of nifedipine on cardiac venous blood flow and myocardial oxygen consumption

CVF Heart rate blood pressure index (HR BP)=heart rate (HR)/min systolic BP=left ventricular pressure work

$$LVW = \frac{\dot{Q}\ (\text{peak BP} - \text{LVFP})\ 13\ 6}{1000}$$

LVFP is in the calculations represented by the mean pulmonary capillary pressure

FFA (assayed according to Dole (3) as modified by Trout et al (19)) uptake=(arterial–cardiac venous con centration) cardiac plasma flow

$$\text{Coronary arteriolar resistance} = \frac{\text{mean BP}}{\text{CVF}}$$

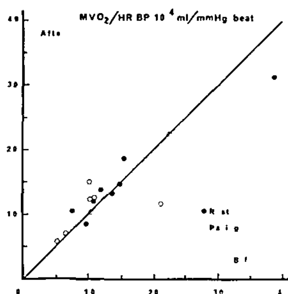


Fig 2 Effect of nifedipine on myocardial oxygen con sumption per unit left ventricular pressure work

$$\text{Peripheral arteriolar resistance} = \frac{\text{mean BP}\ \ 80}{\text{Q}}$$

Statistical analysis was performed using standard procedures Student s *t* test for paired observations was used when measurements before and after nifedipine were compared Differences were regarded as significant when $p<0\ 05$

RESULTS

The effects of nifedipine on coronary and general haemodynamics and FFA are demonstrated in Table I

Coronary haemodynamics

Prior to drug administration there was a propor tional increase in CVF (31%) and MVO_2 (28%) in response to atrial pacing This demonstrated that it was possible to increase blood flow secondary to an increased oxygen demand despite severe coronary arteriosclerotic disease After nifedipine there was a significant (13%) increase in CVF at rest (Fig 1) MVO_2 (Fig 1) and coronary arteriovenous oxygen difference were not significantly altered The rela tion between MVO_2 and HR BP index was also unchanged (Fig 2) demonstrating that the oxygen consumed per unit of pressure work was unaffected by the drug Coronary arteriolar resistance was significantly reduced at rest (11%) (Fig 3) During atrial pacing there was no significant of the parameters

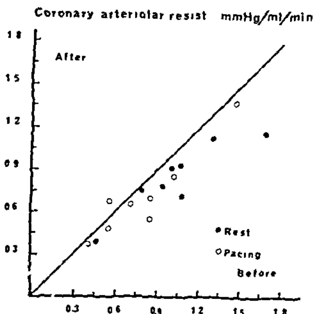


Fig 3 Effect of nifedipine on coronary arteriolar resistance

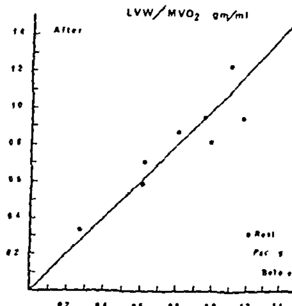


Fig 4 Effect of nifedipine on left ventricular efficiency index

General haemodynamics

At rest and after medication there was a small but insignificant increase in HR The afterload was reduced significantly because of a decrease in peripheral arteriolar resistance which also led to an increased Q and stroke volume The HR BP index was unchanged LVW was increased due to an increase in Q but there was no demonstrable effect left ventricular efficiency (Fig 4) The preload ...sented by the mean pulmonary wedge pressure remained constant

During atrial pacing the peripheral arteriolar resistance decreased after medication whereas Q̇ (Fig 5) and stroke volume increased as at rest All other parameters measured were unaltered

DISCUSSION

The MVO may be influenced by various parameters well known to affect left ventricular oxygen demand Among the most important are HR and afterload or both combined in the double product the HR BP index (11) In addition there are factors such as preload contractility and left ventricular dimensions (12)

In the present study the resting HR was virtually unaffected by nifedipine while the afterload was significantly reduced at rest but not during the stress test Experimental studies have shown that nifedipine tends to increase HR (6 7) while Becker et al (1) concluded that neither resting nor exercise HR was influenced by the drug The reduced resting BP observed would facilitate heart work and improve cardiac energy balance As the oxygen consumption per unit of rate pressure index remained constant this demonstrates that there was no net effect It was merely a matter of adjusting the oxygen supply to the requirement Submitted to the same stress test before and after nifedipine the situation was the same The oxygen consumption per unit of rate pressure index remained constant

Pressure work load (HR BP) is considered to be more oxygen-consuming than volume work load (LVW) (2) In the present study the reduction in peripheral arteriolar resistance with an increased Q̇ led to an increased LVW at rest For another C⁺ antagonist verapamil this has been found to give an increased left ventricular efficiency in animal experiments (10) which was not demonstrable in patients with IHD after nifedipine administration

Our results agree with those of previous studies (7) in that it was obvious that nifedipine had no influence on preload The effect on left ventricular dimensions and contractility was not examined by us but these parameters have previously been found to be unaffected by the drug (7 14) Thus nifedipine does not affect MVO via these factors

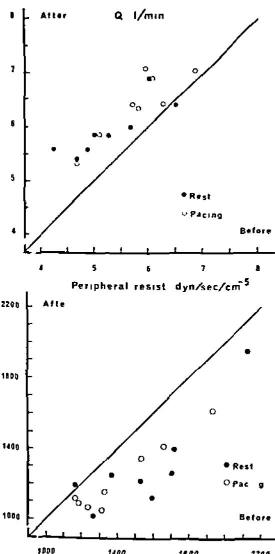


Fig 5 Effect of nifedipine on cardiac output and peripheral arteriolar resistance

Coronary dilatation in excess of that necessary for *adequate* MVO_2 *will* give an increased flow and both a decreased arteriolar resistance and arteriovenous oxygen difference We found a significant decrease in coronary arteriolar resistance and an increased flow at rest but not during atrial pacing Combined with a disproportionate increase in MVO the resting observation at least gives evidence of a coronary vasodilatory effect Nifedipine must therefore be regarded as a coronary vasodilator in humans and has the same properties as other CA^{++} antagonists (8)

Our findings of an insignificant reduction in coronary resistance when the patients were close to their angina threshold may indicate that the vasodilatory effect is weak or that the coronary artery disease in our patients was too severe to permit further dilation of the vessels (7) This agrees in general with the effects observed after verapamil (17) but is in contrast with the finding of an increased coronary arteriolar resistance described after β blockade at rest (16)

A reduction of myocardial oxygen requirement may also be brought about by interfering with cardiac metabolism High arterial concentrations of FFA increase the uptake in the myocardium thereby increasing MVO_2 (15) As FFA metabolism was unaffected by nifedipine this mechanism does not seem to be of importance which is in accordance with experimental data obtained in the dog (9)

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Renin Release in Relation to Plasma Noradrenaline during Supine Exercise in Cardiac Patients

Birger Hesse Niels Juel Christensen and Ellen Damgaard Andersen

From the Cardiovascular Laboratory Medical Department B Rigshospitalet Medical Department F Herlev Sygehus Copenhagen and the 2nd Clinic of Internal Medicine Kommunehospitalet Århus Denmark

ABSTRACT Plasma renin activity in the brachial artery and in the renal vein together with arterial noradrenaline concentration were determined repeatedly during and after 10 min of dynamic exercise in eight cardiac patients Arterial renin increased slightly during exercise and gradually returned to control level after exercise Renal vein renin increased markedly during exercise and returned to the resting value immediately after exercise similarly to the changes in plasma noradrenaline and heart rate A close temporal relationship between the changes in the renal veno-arterial renin difference plasma noradrenaline and heart rate strongly suggests that the sympathetic nervous system is a major determinant of renin release in man during exercise

A large number of experimental and clinical data have supported adrenergic renin release 1) In experimental animals stimulation of the sympathetic renal nerves causes renin release (4 18 25) 2) The juxtaglomerular apparatus is richly innervated (2) 3) Catecholamine administration to the isolated kidney leads to increased plasma renin activity (PRA) (17 26) 4) Beta blockade both acute and chronic has been found to reduce PRA in man in the resting supine condition and following several stimulatory procedures (3 22 23) However a direct close relationship between signs of increased sympathetic nervous activity and renin release in physiological conditions in man has never been reported

If sympathetic nervous activity is a factor of importance for renin regulation it should be possible to demonstrate this relationship during physical exercise a situation in which increased sympathetic nervous activity is a predominant feature The purpose of the present study was to examine the relationship between arterial plasma noradrenaline concentration and PRA in the renal vein and the brachial artery during rest and physical exercise The opportunity was taken to catheterize the renal vein in patients undergoing heart catheterization for diagnostic purposes

PATIENTS

Eight patients referred for hemodynamic investigation were included in the study Age sex diagnosis and functional classification according to the criteria of New York Heart Association are listed in Table I The patients treated with diuretics received potassium supplementation All were on liberal salt and water intake Informed consent was obtained in each case

PROCEDURE

A right heart catheterization was performed in the morning The patients received a light breakfast but no medication apart from 200 mg phenobarbitone as sedation one hour prior to the hemodynamic examination

A Lehman catheter no 6 was inserted into the left brachial vein and the tip was placed in the main stem of the pulmonary artery A polyethylene catheter 20 cm long was placed in the left brachial artery by the Seldinger technique A polyethylene catheter no 6 was introduced via a superficial vein from the left arm into the left renal vein with the tip placed at the hilum of the kidney

After pressure recordings and measurement of cardiac output at rest a bicycle ergometer (Siemens Elema Sweden) was connected to the catheterization table Submaximum supine exercise was performed for 10 min at loads varying from 50 to 300 kpm/min according to the clinical impression of the patient s exercise capacity and the pressure level in the pulmonary circulation The load often had to be adjusted during the first 2 min according to the pressure increase in the pulmonary artery After termination of the exercise test the patients rested for 20 min with the catheters still in the same positions Blood samples for plasma catecholamines in the brachial artery and PRA in the brachial artery and renal vein were drawn before after 5 and 10 min of exercise and 5 10 and 20 min after termination of the exercise

Heart rate brachial and pulmonary artery and renal vein pressures were recorded at the same intervals and

Requests for reprints B Hesse Dept of Clinical Physiology Glostrup Hospital DK 2600 Glostrup Denmark

Table I *Clinical data on the patients*

Pat no	Age (y)	Sex	Diagnosis	Medical treatment	NYHA class
1	56	♀	Mitral stenosis and regurgitation	Furosemide 120 mg	II
2	54	♀	Mitral stenosis and regurtitation	Bumetanide 2 mg digoxin 0 188 mg	II
3	55	♂	Mitral stenosis and regurgitation	Bumetanide 2 mg digoxin 0 125 mg	II
4	19	♀	Aortic incompetence		II
5	50	♀	Mitral valve prosthesis	Furosemide 80 mg digoxin 0 25 mg	II
6	53	♀	Mitral valve prosthesis	Bumetanide 2 mg digoxin 0 25 mg	III
7	43	♀	Cardiac neurosis	Hydroflumethiazide 50 mg	II
8	52	♀	Mitral stenosis and aortic incompetence	Furosemide 80 mg digoxin 0 188 mg	III

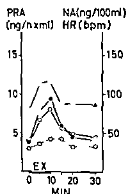
pulmonary artery oxygen saturation was measured Expired air for determination of cardiac output was collected from the 2nd to the 5th min of exercise according to the routine of the laboratory

METHODS

Pressures were measured with capacitance transducers (14) and recorded together with the ECG on a U V recorder (SE 3012 SE Laboratories England) Cardiac output was measured by the direct Fick principle from duplicate pulmonary and brachial arterial blood samples Oxygen content of the blood was determined with an oximeter (Siemens Elema Sweden) and Hb with a photoelectric hemoglobinometer (Haemotest Testa Laboratories Denmark) Expired air was collected into Douglas bags at rest over 5 min and during exercise over 3 min Oxygen content was measured by Haldane analysis and volume by a gasometer Plasma noradrenaline and adrenaline con

Table II *Measured values before and during exercise*
R=rest before exercise E=at 10 min of exercise

		Mean of paired values	Range	No of pairs	*p*
Brachial artery systolic pressure (mmHg)	R	144	120–190	8	<0 01
	E	179	148–285		
Brachial artery diastolic pressure (mmHg)	R	75	55–93	8	<0 05
	E	84	73–99		
Renal perfusion pressure (mmHg)	R	90	75–108	8	<0 01
	E	104	84–152		
Pulmonary artery mean pressure (mmHg)	R	23	13–32	8	<0 01
	E	37	18–54		
Cardiac index (l/min×m²)	R	2 4	1 5–4 2	7	<0 02
	E	4 1	2 2–6 5		
Oxygen uptake (ml/min)	R	179	148–248	7	<0 02
	E	580	380–862		
Heart rate (bpm)	R	78	66–96	8	<0 01
	E	118	84–156		
Arterial adrenaline concentration (ng/100 ml)	R	8	3–18	7	>0 10
	E	10	5–26		
Arterial noradrenaline concentration (ng/100 ml)	R	38	13–52	7	<0 02
	E	94	24–179		
Arterial plasma renin activity (ng×h⁻¹×ml⁻¹)	R	3 0	1 4–5 9	8	<0 01
	E	4 2	1 5–9 0		
Renal vein plasma renin activity (ng×h⁻¹×ml⁻¹)	R	3 9	2 4–7 9	8	<0 01
	E	8 1	3 6–16 0		



(Fig 1 Mean values of plasma renin activity (*PRA*) in the renal vein (O—O) and brachial artery (O---O) plasma noradrenaline concentration (●) and heart rate (▲) during and after exercise (*EX*)

centrations were determined by a double isotope derivative technique (6 11)

PRA was measured by a radioimmunoassay as described by Haber et al (13) with the Angiotensin I [125]I kit of NEN (New England Nuclear) The plasma samples from each patient were all measured in the same analysis The coefficient of variation of samples run in the same analysis is in our laboratory 3 3% The normal range of PRA in peripheral (venous or arterial) blood in our laboratory is 0 67–? 90 ng × h⁻¹ × ml⁻¹

Results were evaluated statistically by linear regression analysis and the Wilcoxon test for paired differences (10)

RESULTS

At rest five of the patients had a low cardiac index (≤2 4 l/min×m²) and five had elevated pulmonary artery pressures Arterial pressures were normal in all but one patient (no 8) with aortic incompetence

During exercise brachial and pulmonary artery pressures cardiac index and heart rate increased (Table II) The exercise loads were comparatively small hence the modest increase in oxygen uptake

Arterial PRA was elevated in three patients at rest It increased significantly during exercise on average by 40% Renal vein PRA increased markedly during exercise on average by 140% (Table II) Arterial plasma noradrenaline increased significantly during exercise whereas plasma adrenaline was unchanged (Table II)

When either all arterial or all renal vein PRA values are correlated with the noradrenaline values rather poor correlation coefficients are found (arterial PRA $r=0$ 15 $p>0$ 10 renal vein PRA $r=0$ 32 $p<0$ 05) But a temporal relationship is evident between renal vein PRA plasma noradrenaline and heart rate (Fig 1) The peak values of the three variables occur at 10 min and each of them falls steeply from the 10th to the 15th min By contrast the arterial PRA attains its maximum at 15 min with a slight increase from the 10th to the 15th min Fig 2 shows a similar relationship between plasma noradrenaline heart rate and the changes in the veno arterial PRA difference over the kidney divided by the arterial PRA

In two of the patients (nos 3 and 4) the connection between plasma noradrenaline concentration and the PRA values of the renal vein is further supported by regression analysis which reveal significant correlations but different slopes Fi In most of the remaining patients the values e clustered in two groups with low and high values respectively rendering statistical analysis meaningless

DISCUSSION

This study demonstrates a marked difference between the arterial and renal vein PRA responses during exercise The mean rise in the renal vein PRA was more than three times the increase in arterial PRA and the temporal relationships were different the time course of the renin changes in the renal vein were closely related to the changes in plasma noradrenaline and heart rate whereas the arterial renin increase was more delayed and sustained

A rise in arterial PRA during exercise does not necessarily indicate an increase in renin release since it may be influenced by changes in substrate concentration and changes in the elimination rate of

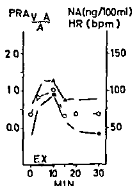


Fig 2 Mean values of plasma noradrenaline concentration (●) and heart rate (▲) in relation to the mean changes in the veno-arterial PRA difference over the kidney divided by the arterial PRA (O)

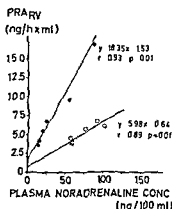


Fig 3 Plasma renin activity of the renal vein (PRA_{RV}) correlated to plasma noradrenaline concentration in patient 3 (O) and patient 4 (●)

renin But either possibility appears unlikely (12 15)

The renal vein PRA as well as the renal veno arterial PRA difference may be influenced by another factor the renal blood flow From the rise in arterial PRA we known that renin release is increased Although some reduction of the renal blood flow may well occur during exercise the time course of the increase in renal vein PRA probably reflects the time course of the increase in renin release It is emphasized that the increase is limited to the exercise period and that PRA of the renal vein rapidly returns to the resting level after exercise thus closely following the noradrenaline pattern

There is a large body of evidence indicating that plasma noradrenaline concentration reflects the level of sympathetic nervous activity also in a state of sodium depletion (8 20) Accordingly it can be stated that the increased renin release during exercise in well compensated cardiac patients is temporally closely related to a reliable index of the sympathetic nervous activity This suggests a causal role of the sympathetic nervous system for increased renin release during exercise

The presence of cardiac failure might cause renin suppression but this should be counteracted by diuretic treatment (16) Hence the sensitivity of the renin system to sympathetic nervous stimulation in our patients should not be suppressed This is confirmed by comparison of the peripheral PRA response with other studies of moderate exercise in normal subjects Thus near maximal supine exercise for 10 min in normal subjects caused a small increase in peripheral PRA smaller than the changes observed in serial samples taken at rest on other occasions (7) Kotchen et al (19) found no increase in PRA in normal subjects performing 10 min exercise at 40% of $V_{O_2\,max}$ in the sitting position but a 3 5 fold increase at 70% of $V_{O_2\,max}$ After 5 min of moderately heavy exercise in the sitting position PRA had increased by 46% in 7 normal subjects ($p>0\ 05$) after 30 min by 132% ($p<0\ 01$) (24) During 7–10 min strenuous exercise no increase in peripheral PRA occurred in normal conscious dogs (21)

The increase in renin release during moderate exercise may seem surprisingly small in relation to the importance of the sympathetic nervous system for renin regulation recently proposed (9 27)

However two points should be taken into consideration firstly the sympathetic stimulation of renin release during exercise may be counteracted by an increased renal perfusion pressure inhibiting the renal baroreceptor Secondly the rather short duration of renin stimulation in most exercise studies should be noted being interrupted at the moment exercise is stopped as shown in the present study In prolonged exercise marked PRA increases were observed (5) Other kinds of renin stimulation last considerably longer (posture diuretics thermal stress, sodium restriction)

It is concluded that the sympathetic nervous system has a definite but modest influence on renin release in compensated cardiac patients being of major importance for the renin increase during exercise The individual differences in the renin response to exercise at the same plasma noradrenaline level (Fig 3) are probably largely due to differences in sodium balance (1)

ACKNOWLEDGEMENTS

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Dialysis Fistulas and Cardiac Failure

Harald Bergrem Audun Flatmark and Svein S monsen

From the Med cal Department B and Surg cal Department B Un ers ty Hosp al R kshosp talet Oslo Norway

ABSTRACT A haemodialysis fistula may be a factor in the development of cardiac failure and surgical reduction of fistula blood flow can improve the condition We have investigated 6 patients with dialysis fistulas and closed the fistula in one of them with marked improvement of cardiac failure It is unlikely that a peripheral dialysis fistula can cause cardiac failure by itself but it may be a contributing factor when the cardiac reserve is reduced due to other causes

Arteriovenous (AV) fistulas usually of traumatic origin may cause card ac failure—especially when located to the central vessels (7) Surgical closure of the f stula may improve or cure the cond tion

Blood access for chron c haemodialysis is obtained either by an external AV teflon shunt (8) or a subcutaneous AV fistula (3) Fistulas are usu ally preferred because compl cations such as coagulation and infection are less common than w th the teflon shunt (4 9) Dialysis fistulas are preferably created on the distal forearm with an optimal length of 6–9 mm

Over the last few years several reports have documented that dialysis fistulas may contribute to the development of card ac failure in d alysis pa t ents Surgical intervention in order to reduce blood flow may improve the condition (1 2 5 6) These reports prompted the present haemodynamic study of 6 patients with large fistulas and/or cardiac symptoms

PATIENTS

S x patients aged 32–64 years were stud ed Pert nent cl nical data are summarized in Table I All had an AV f stula of 4–58 months duration d stally on the forearm Three pat ents were treated with regular haemod alys s while 3 had pers stent f stulas 17 3 and 48 months after successful renal transplantat on Two had cl n cally large f stulas without accompanying card ac symptoms wh le 4 had card ac failure and card omegaly All had normal BP at the time of investigat on

METHODS

A right s ded card ac catheterizat on as per the right femoral ve n In 3 patients we u ed C catheter no 7 and determined the card a output a ing to the method of Fick In the o her 3 a Swan floating catheter was used and card ac output was m e ured by thermod lut on technique Intra ard ac press were measured on an EMT transducer (E ema Schor n der) and recorded on a Mingograph 800 ES) d rect writ ng ink jet recorder The fourth interc stal spa e in the anterior axillary l ne was used for ze o refe ence Mea urements were made under rest ng cond t ons 10 m n after the catheter had been pos t oned and repeated 5 m n after a sphygmomanometer cuff had been inflated to a pressure 30 mmHg above the systol c BP just prox mal to the f stula

RESULTS

The resting cardiac output was greatly increased in pat ents 1 and 3 and fell by 1/3 when the fistula was temporarily occluded Patient 5 had a correspond ing fall of 25% while the other three patients had only a small drop in cardiac output after occlus on There were no sign f cant changes in heart rate or intracard ac pressures The pulmonary capillary pressure in patients 1 and 6 was increased the other pressure read ngs were normal (Table II) In one patient with increasing symptoms of cardiac failure the fistula was closed with a striking effect on the symptoms

CASE HISTORY

Pat ent 5 a woman aged 50 had received a f stula but had not been d alyzed when she was successfully transplanted w th a cadaver k dney on Feb 10 1977 One month after the operat on she developed peripheral oedema and in creas ng dyspnoea Card ac catheterizat on showed a sl ghtly increased card ac output (6 7 l/m n) w th a drop of 24 2% when the f stula was occluded Her card ac vol ume as judged by X ray had increased from 560 ml/m² after the transplantat on to 950 ml/m² in July 1977 when the f stula was closed permanently The ankle oedema d sappeared w th n 24 hours and her dyspnoea improved rapidly flu d ntake and med cat on remain n

Table I *Clinical data on 6 patients with AV dialysis fistulas*

Pat no	Age (y)	Age of fistula (mo)	Dialysis	BP (mmHg)	Heart size (ml/m² BSA)	Clinical heart failure	Clinical fistula size
1	66	15	Yes	150/70	700	+	Large
2	41	4	Yes	160/80	1 080	+	Small
3	39	56	Yes	140/70	340	–	Large
4	60	30	No	115/70	420	–	Large
5	50	8	No	140/70	560	+	Large
6	63	58	No	135/85	660	+	Large

Patients 4 5 and 6 had a functioning fistula 17 3 and 48 months after successful renal transplantation

Table II *Cardiac output heart rate and intracardiac pressures*

A=fistula open B=fistula occluded AP=mean pulmonary artery pressure PCV=pulmonary capillary wedge pressure

Pat no	Cardiac output (l/min)		Difference		Heart rate (beats/min)		AP (mmHg)		PCV (mmHg)	
	A	B	l/min	%	A	B	A	B	A	B
1	10 2	6 8	3 4	33	70	68	26	27	19	21
2	5 4	5 2	0 2	< 5	90	90	25	26	8	8
3	15 0	10 2	4 8	32	75	73	11	10	7	6
4	6 2	5 6	0 6	10	66	63	11	11	4	5
5	6 7	5 2	1 5	24	88	86	25	22	8	8
6	4 0	4 1	0 1	< 5	78	78	27	26	22	21

One and a half months later she had no oedema and only slight breathlessness during moderate exercise Her heart size had not decreased during this period and she has had a few episodes of paroxysmal atrial fibrillation She has also developed a heart murmur of mitral regurgitation

DISCUSSION

Although it has been demonstrated that dialysis fistulas may contribute to the development of cardiac failure the present and other investigations clearly show that the change in cardiac output varies from patient to patient and that the development of cardiac failure bears relatively little relation to the fall in cardiac output when the fistula is temporarily occluded Some patients have a greatly increased cardiac output with a significant drop during occlusion (32% in our patient 3) without any clinical or radiological signs of cardiac failure

This discrepancy between cardiac output and clinical fistula size on the one hand and the development of cardiac failure on the other is undoubtedly an expression of the myocardial reserve capacity of the individual patient Patients are especially at risk if they have had previous episodes of cardiac failure, long standing hypertension and/or coronary insufficiency (2) Our patients 2 5 and 6 had previously been treated for hypertension

If a properly dialyzed patient develops cardiac failure the primary management should be conservative If this alone is not successful a patent dialysis fistula must be born in mind as a contributing factor and surgical reduction of fistula blood flow or change to an external teflon shunt should be considered In a transplanted patient with stable graft function permanent closure of the fistula is the treatment of choice If however the patient has no symptoms of cardiac failure and the heart is of normal size there is no basis for routinely closing a patent dialysis fistula after a successful transplantation

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Hemodynamic Effects of the Cardioselective β-Blocking Agent Metoprolol in Acute Myocardial Infarction

A 24 Hour Catheterization Study

Ivan Malek [1] Finn Waagstein Åke Hjalmarson Stig Holmberg
and Karl Swedberg

From Department of Medicine I Sahlgren s Hospital University of Goteborg Goteborg Sweden

ABSTRACT Hemodynamic changes were studied in ten patients with uncomplicated transmural myocardial infection during 24 hours on β blockade The cardioselective β adrenergic blocking drug metoprolol was injected (15 mg i v) within the first 24 hours after onset of chest pain and was followed by oral therapy (25–50 mg at 6-hour intervals) There was a decrease in heart rate, systolic BP, and cardiac output, which was most marked after the injection The stroke volume and diastolic BP for the whole group of patients remained unchanged The pulmonary artery end diastolic pressure did not change significantly after the injection but a continuous fall was obtained in three out of four patients with initially elevated values The preejection period, measured from the ECG and carotid pressure curve, was initially short and was prolonged in all patients after administration of the β blocking drug It is concluded that the cardioselective β blocking drug metoprolol may be used in selected patients in the acute phase of myocardial infarction without danger of hemodynamic deterioration during the first 24 hours of therapy The selection of patients can be based on clinical criteria In this study signs of left heart failure hypotension poor peripheral circulation bradycardia and AV block were regarded as contraindications

There is clear evidence that myocardial infarction in man develops in a stepwise manner (2 14 18) The final extent of necrosis depends mainly on the balance between metabolic demand coronary supply and washout of metabolites (7) Many factors might influence the outcome in primarily ischemic zones especially in the border zone where a considerable coronary flow may still be present (24) One of the most important is the high level of sympathetic activity The hemodynamic and metabolic consequences of the increased sympathetic stimulation together with the release of endogenous noradrenaline from the nerve endings in the heart (29) considerably increase myocardial oxygen consumption and the degree of ischemia The use of β adrenergic blocking drugs is thus a logical therapeutic approach The injection of a β blocker in the acute phase of myocardial infarction has undoubtedly a favorable effect on the degree of ischemia (15 16 21 26 27 28) The positive clinical effects of the administration of β blocking drugs in acute myocardial infarction are manifested as a reduction in chest pain and in ventricular arrhythmias (10 26 27 28) These favorable acute effects are obtained without serious hemodynamic deterioration especially since there was no increase in the filling pressure of the left ventricle (9 15) or fall in stroke volume in a selected group of patients without signs of heart failure The long term hemodynamic effects of β adrenergic blocking drugs have not been studied however

The aim of this study was to follow the hemodynamic changes in patients with acute myocardial infarction during their first 24 hours on the cardioselective β blocking agent metoprolol (1)

Address for reprints Å Hjalmarson M D Department of Medicine I Sahlgren s Hospital S-413 45 Göteborg Sweden

[1] *Present address* 2nd Research Unit of Internal Medicine Institute of Clinical and Experimental Medicine Prague 4 Krč Czechoslovakia

Abbreviations BP blood pressure ECG=electrocardiography AV=atrioventricular AMI acute myocardial infarction ASAT=aspartate aminotransferase alanine aminotransferase HR=heart rate

Table I *Age location of myocardial infarction (MI) $ASAT_{max}$, $ALAT_{max}$, body temperature$_{max}$ and heart size during the first three days after mobilization*

A=anterior I=inferior Normal value for ASAT and ALAT ≤0 7 μkat/l normal heart size ≤450 ml/m² BSA

Pat no	Age (y)	Location of MI	$ASAT_{max}$ (μkat/l)	$ALAT_{max}$ (μkat/l)	$Temp_{max}$ (C)	Heart size (ml)/ (ml/m²)
1	67	A	2 5	0 4	38 4	770/400
2	68	I	2 4	0 4	37 9	Normal
3	60	A	3 7	0 6	37 8	790/470
4	54	I	2 3	1 2	37 8	1 020/650
5	67	A	2 2	0 2	37 7	1 150/600
6	60	I	2 9	0 7	38 4	870/520
7	68	A	3 8	0 8	38 8	–
8	55	A	6 3	1 2	39 4	970/510
9	69	A	1 8	0 7	37 7	880/470
10	53	A	8 0	2 2	39 0	1 080/560

PATIENTS AND METHODS

Ten male patients with AMI were studied within 24 hours after onset of clinical symptoms The diagnosis was based on a typical history development of new Q waves on the ECG recordings and transient increase in ASAT values Table I summarizes the clinical data All the patients were in functional class I (11) They had sinus rhythm with a PQ interval of less than 0 20 sec and none had a heart rate below 50 beats/min or a systolic BP below 100 mmHg

A Swan-Ganz thermodilution catheter no 7 was introduced into the pulmonary artery via a cubital vein The catheter was attached to a Statham P 23dB transducer connected to a Honeywell ACC 113 amplifier The thermodilution method was used for measurements of cardiac output (Cardiac Output Computer 3750 Car ascular Instruments London) Sodium chloride ml) at room temperature was injected into the catheter the thermodilution curves were recorded with the on a direct writing recorder (Mingograph 34 lema Schonander Sweden) at a paper speed of 5 mm/sec Only proper thermodilution curves were accepted The mean of two values of cardiac output was used for each measurement The difference never exceeded 10% The systemic BP was measured indirectly by auscultation or by the ultrasonde method (Arteriosonde 1217 Hoffman La Roche Cranbury USA) (8) The mean of three values during the last 10 min of the control period and the mean of two values during each registration period were used The peripheral vascular resistance was calculated from the mean arterial pressure and cardiac output (mean arterial pressure/cardiac output) The systolic time intervals were calculated from simultaneous recordings of ECG lead II phonocardiogram and carotid artery pressure curve (28) The values of the preejection period and left ventricular ejection time were corrected for HR (30)

Metoprolol (Seloken® Hassle Sweden) a cardioselective β-blocking drug (1) was injected into the peripheral vein in doses of 5 mg at 2-minute intervals up to a full dose of 15 mg. Oral medication was started 30 min after the injection and repeated at 6-hour intervals The daily oral dose used was 100 mg in two patients and 200 mg in eight patients

All measurements were done during the last 10 min of the control period 20–30 min after the injection and repeated at 6-hour intervals during 24 hours (Fig 1)

The paired *t* test was used for the statistical analysis of the results A *p* value <0 05 was considered statistically significant.

RESULTS

Clinical findings

All ten patients tolerated the full i v dose of 15 mg metoprolol In two cases (nos 5 and 8) the systolic BP decreased below 100 mmHg within 30 min after the injection (Fig 2) These patients were therefore given an oral dose of 25 mg four times daily None of the patients showed signs of compromised circulation One patient (no 7) developed rapid atrial fibrillation approximately 20 hours after the injection this was subsequently converted to sinus rhythm by digitalization None of the patients developed signs of pulmonary congestion poor peripheral circulation or AV block

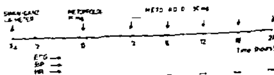


Fig 1 Time schedule for administration of metoprolol and hemodynamic measurements —=Hemodynamic measurements (noninvasive pulmonary artery pressures cardiac output)

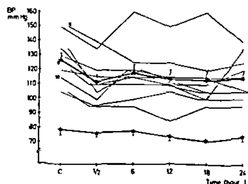


Fig 2 Changes in systemic BP in 10 patients with AMI during 24 hours on metoprolol Individual values and mean values are given for the systolic BP and mean values for the diastolic BP All values are means ±S E The mean systolic value was significantly decreased from 1/2 to 24 hours after administration of metoprolol while the diastolic BP was significantly reduced only at 12 and 18 hours The *p* values are given in Table II *C*=control

Hemodynamic changes

The hemodynamic data are summarized in Table II HR decreased in all patients after injection of metoprolol (Fig 3) The change was most marked in the patients with initially elevated values and in these the HR was kept at a reduced value during the whole 24-hour period The systolic BP fell in all patients after the injection (Fig 2) In two patients a fall below 100 mmHg was observed without any signs of poor peripheral circulation A small decrease in the diastolic BP during 12 and 18 hour periods was obtained Thermodilution curves of good quality were registered in eight patients during the whole period The cardiac output decreased after injection in all cases and the mean value remained reduced except during the last period measured (Fig 4) The stroke volume increased in three patients and decreased in five (Fig 5) The mean value remained unchanged during the period of measurement The very small but significant increase in the peripheral vascular resistance was seen 30 min after the i v injection of metoprolol but no changes compared to the control value were observed 6–24 hours after the injection (Table II)

The pulmonary artery end diastolic pressure was initially elevated above 12 mmHg in four patients and remained within normal limits in six patients (Fig 6) The pressure decreased continuously during 24 hours in three of the patients with initially high values In one patient (no 10) the pulmonary artery end diastolic pressure remained elevated after the completion of the study and fell after injection of 40 mg furosemide from 20 2 to 15 6 mmHg The initial mean value of the pulmonary artery end diastolic pressure was 12 1±2 0 mmHg and did not change significantly during 24 hours

Table II *Hemodynamic values during 24 hours on metoprolol (means ±S E range in parentheses)*

	No of pats	Control	Hours after injection of metoprolol				
			1/2	6	12	18	24
HR (beats/min)	10	76±4 9	65±3 6	67±2 9*	62±2 4*	65±2 1	68±2 9
Systolic BP (mmHg)	10	127±5 1	112±4 9 **	118±5 6	114±5 2 *	113±5 7	115±4 6
Diastolic BP (mmHg)	10	79±3 6 (70–100)	77±3 3 (60–100)	79±2 7 (65–95)	73±2 9 (55–90)	71±2 9 * (60–90)	74±2 4 (65–90)
Pulmonary artery end diastolic pressure (mmHg)	10	12 1±2 0	13 3±2 0	13 5±2 0	11 7±1 6	11 1±1 3	11 0±1 1
Cardiac output (l/min)	8	5 2±0 2	4 3±0 2**	4 6±0 1 *	4 3±0 1 *	4 5±0 1	4 7±0 3
Stroke volume (ml)	8	66±3 1	65±3 2	66±2 8	69±3 8	70±3 7	69±2.3
Peripheral vascular resistance (u)	8	18 2±0 8 (14 3–21 2)	20 5±0 9 (15 4–23 8)	20 0±0 4 (18 7–21 8)	20 4±0 7 (17 0–22 6)	18 3±1 0 (14 4–24 1)	19 0±1 2 (15 8–24

Significance of differences between control and metaprolol values p<0 05 p<0 01 ***p<0 001

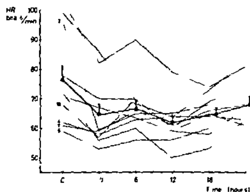


Fig 3 Changes in heart rate in 10 patients with AMI during 24 hours on metoprolol Individual values as well as the means ±S E are given HR was significantly reduced at 1/2 to 18 hours after administration of metoprolol The *p* values are given in Table II *C*=control

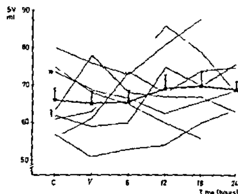


Fig 5 Changes in stroke volume in 8 patients with AMI during 24 hours on metoprolol Individual values and the means ±S E are given There were no significant changes after administration of metoprolol *C*=control *SV*=stroke volume

Noninvasive variables

The changes in the noninvasive variables are listed in Table III and Fig 7 Carotid artery pressure curves of good quality were obtained in nine patients The preejection period index was initially shortened in six cases and within normal limits (30) in three patients A prolongation of the preejection period index was obtained in all nine patients at 30 min after the injection of a β blocking drug The mean value for the group remained significantly higher after 6 12 and 18 hours The left ventricular ejection time index did not change consistently and the mean value remained unchanged during the period of measurement Preejection period/left ventricular ejection time followed changes in the preejection period index

DISCUSSION

The acute hemodynamic effects were characterized by a decrease (approximately 15%) in HR systolic BP and cardiac output and an increase in peripheral vascular resistance (13%) Jewitt et al (9) found similar changes after 25 mg of practolol i v On the other hand a marked increase in peripheral vascular resistance and a fall in cardiac output were observed after 5 mg of propranolol i v (3) The importance of the cardioselectivity of a β blocker

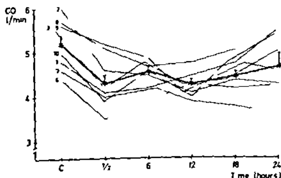


Fig 4 Changes in cardiac output in 8 patients with AMI during 24 hours on metoprolol Individual values as well as the means ±S E are given The cardiac output was significantly reduced at 1/2 to 18 hours after administration of metoprolol The p values are given in Table II *C*=control *CO*=cardiac output

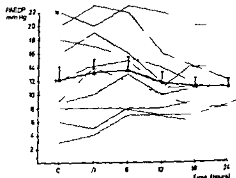


Fig 6 Changes in pulmonary artery end diastolic pressure in 10 patients with AMI during 24 hours on metoprolol Individual values and the means ±S E are given No significant changes were seen after administration of metoprolol *C*=control *PAEDP*=pulmonary artery end diastolic pressure

Table III *Preeject on per od index (PEPI) left ventr cular eject on t me ndex (LVETI) and PEP/LVET rat o in n ne pat ents d r ng 24 hours on metoprolol (means +S E range n parentheses)*

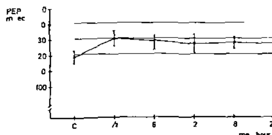
	Control	Hours after nject on of metoprolol 1/2	6	12	18	24
PEPI (m ec)	119 4+4 2 (97 134)	131 0+5 1** (112 157)	129 6+5 9** (118–167)	127 5+5 9* (98–159)	1 8 2+3 8* (109–147)	127 1+4 6 (113–148)
LVETI (msec)	389 3+7 2 (336–412)	390 4+4 4 (374–411)	386 6+5 1 (379–410)	384 1+4 2 (369–399)	385 6+3 5 (375–406)	390 5+3 6 (378–405)
PEP/LVET	0 336+0 00	0 376+0 002**	0 375+0 003	0 368+0 00?*	0 371+0 00 *	0 372+0 00?

S gn f cance of d ffe en es bet veen control and me op olol values *$p<0\ 05$ **$p<0\ 01$

used in pat ents w th AMI and a high level of sympathet c activ ty can be deduced from these results Mueller (15) however found only a sl ght decrease in BP and card ac output in pat ents w th AMI w thout any changes n peripheral vascular res stance after 0 1 mg of propranolol/kg b wt The present 24 hour mon toring study showed that the most marked decrease n card ac performance was obtained after i v inject on of a β blocking agent Thus inject on of the drug can be used as a therapeut c test before the pat ent is put on con t nuous oral treatment In fa t the daily oral dose n our pat ents was adjusted accord ng to the BP reac tion 30 m n after the njection Furthermore it was shown in the present study that the long term changes in pulmonary artery pressures can be s m lar to those in a group of pat ents without compl ca tions who are not treated with β block ng agents Hemodynamic mon toring in th s group (functional class I) (11) nd cated that the elevat on of the left ventricular end d astol c pressure w th subsequent pulmonary hypertens on is present in the early phase of the d sease n many nstances (5 12 19) A continuous fall during the follow ng days is the usual p cture (13 20) A decrease in the pulmonary artery end d astol c pressure was obta ned in this study n three out of four pat ents w th in t ally elevated values

The t me course of the elevated f ll ng pressure cannot be pred cted from the beg nn ng and n some of the n t ally uncompl cated cases s gns of pulmonary edema m ght develop later even w th out adm n strat on of β block ng drugs These pa tients are characterized by the pers stence of an elevated f ll ng pressure during the f rst day after the onset of chest pa n (13 20) and should obv ously be treated w th d uret cs before s gns of heart failure appear Th s was the case in one of our patients (no 10) in whom the elevated f lling pres sure was an nd cat on for d uret c treatment A decrease n fill ng pressure without changes n card ac output and systemic BP was obtained after 40 mg of furosem de i v and no s gns of pulmonary congest on developed

Shortening of the preeject on period during the acute phase of an uncompl cated myocard al infarc tion has been observed previously (4) Th s f nd ng is considered to be a consequence of a high level of sympathet c act v ty wh ch s often present in these pat ents (25) However in th s study the mean value of the preeject on period index (119 msec) is lower than has been reported for comparable groups of pat ents (10) The add t onal stress effect of card ac catheterizat on cannot be excluded The rise of the left ventricular end d astol c pressure is known to alter the durat on of the preejection period Both shorten ng (22) and prolongat on (6) have been de



F g 7 Preeject on period ndex n 10 pa en s w h AMI during 24 hours on metop olol The l nes nd cate the mean value and uppe and lower l m ts for normal sub-jects The n tial mean value s shortened compared to normal controls and was s gn f cantly prolonged at 1/2 to 18 hours afte adm n s rat on of metop olol The *p* values are given n Table III All values are means +S E C t ol *PEPI* preejection period ndex

scribed The prolongation of the preejection period index without changes in preload and afterload is interpreted as a consequence of decreased con tractility of the left ventricle This was found after the injection of metoprolol when the prolongation occurred without changes in the pulmonary dia stolic and arterial diastolic pressures Repeated measurements of systolic time intervals during the first few days after onset of chest pain in patients with myocardial infarction have been carried out by Waagstein et al (28) A trend towards prolongation of the preejection period index was seen during the first five days, probably due to decreased sympathetic activity No significant changes be tween the first and second days were observed In our study the preejection period index remained prolonged during the first 24 hours on β blockade indicating that the sympathetic hyperactivity was effectively reduced by the β blockade This find ing is more difficult to interpret however since marked changes in preload (the pulmonary artery end diastolic pressure) were obtained in some pa tients during this period (Fig 6)

The present study shows that patients with AMI without clinical signs of left heart failure hypoten sion poor peripheral circulation bradycardia or AV block will tolerate an i v loading dose as well as continued oral treatment with the cardioselective β blocker metoprolol The drop in systolic BP to a level of about 90 mmHg which was seen in two was not considered alarming These pa were characterized by a low initial BP and peripheral circulation which persisted after injection The good tolerance of the β blocking agent together with only a modest decrease in cardiac performance in spite of high doses of the drug in patients with high sympathetic activity can be explained by a concomitant decrease in the de gree of ischemia in the border zone around the infarct The mechanism for this might be a blockade of the effects of locally released noradrenaline (29) which in the presence of the unchanged coronary flow (17) could explain the improved mechanical performance of the previously ischemic zone (23) It seems justifiable to suggest the use of a car dioselective β blocking agent as early as possi ble in patients with severe ischemic chest pain and probable AMI in order to limit the ischemic dam age Long term studies are in progress to evaluate the β blocking drug metoprolol for preservation of the ischemic myocardium in man

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Prognostic Implications of Ventricular Arrhythmias Registered before Discharge and One Year after Acute Myocardial Infarction

N Rehnqvist T Lundman and A Sjogren

From the Department of Medicine Karolinska Institutet at Serafimerlasarettet Stockholm Sweden

ABSTRACT The prognostic weight of ventricular ectopic beats (VEBs) was evaluated in 160 patients discharged after a CCU treated acute myocardial infarction (AMI) and followed for two years VEBs were registered prior to discharge by 6 hours of telemetry (3 hours during daytime including exercise and 3 hours at night) and again one year after the AMI During the first year of follow up, 11 patients died suddenly and 20 suffered reinfarction Sixteen (55%) of these had shown severe VEBs, i e multiform paired R on T, or ventricular tachycardia, as compared to 42 (29%) of the remainder During the second year of follow up eight patients suffered reinfarction and five died suddenly The occurrence of severe VEBs prior to discharge was not of prognostic value for the second year per se but continued to carry prognostic weight for the first plus the second year One year after the AMI the VEB incidence in 122 survivors without reinfarction increased insignificantly from 71 to 78% VEB severity increased in 43% and decreased in 27% and the shift towards severe forms is significant Severe VEBs one year after the AMI carry a prognostic weight for the second follow up year, as 18% of patients with severe VEBs reinfarcted or died suddenly against 5% of those with no or uniform VEBs only Patients who had severe VEBs both prior to discharge and one year later did especially badly

Sudden death continues to account for an important proportion of deaths in ischemic heart disease (IHD) Accordingly considerable attention has been paid to ventricular arrhythmias in IHD (3 4 8 9 14 15 21 26) and recently the occurrence as well as the prognostic significance of postinfarction ventricular ectopic beats (VEBs) was evaluated in this department (18 19 20) Although this study revealed a relationship between predischarge ventricular arrhythmias and extensive myocardial damage severe VEBs per se were found to carry an independent prognostic weight for the first post infaction year in the form of reinfarction and sudden death The occurrence of VEBs was also reinvestigated one year after the index acute myocardial infarction (AMI) in survivors who had not reinfarcted and a moderate but significant worsening in VEB severity was noted (20)

As all patients now have been followed for two years from their index AMI this study has been performed to assess 1) the prognostic significance of VEBs found prior to discharge for the first two post AMI years 2) the prognostic significance of VEBs seen one year after the index AMI and 3) the *prognostic significance of changes in ventricular* arrhythmias when comparing predischarge and 1 year VEBs Separately in 20 patients the yield of 6 hours recording was compared with that of 24 hours for an evaluation of the registration technique

PATIENTS AND METHODS

Design of the study

The study was performed at the Serafimerlasarettet Stockholm AMI criteria and definitions as well as details concerning catchment area and coronary care unit (CCU) routines have been given previously (18 23) A total of 160 consecutive patients below 66 years of age treated in our CCU for an AMI and who at discharge were in sinus rhythm without complete bundle branch block were investigated for VEBs prior to discharge and one year after the AMI After discharge the patients were seen regularly by the authors at the Outpatient Clinic One year after the AMI survivors who had not reinfarcted were readmitted

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Abbreviations IHD=ischemic heart disease VEB(s)=ventricular ectopic beat(s) AMI=acute myocardial infarction CCU=Coronary Care Unit VT tachycardia

Table I *Distribution of various forms of VEBs one year after AMI in relation to predischarge*

Type of VEBs prior to discharge	Type of VEBs one year after AMI ($n=122$)					
	None	Uniform	Multi form	Paired	R on T	VT
None	*11*	12	8	3	0	1
Uniform	9	*15*	11	5	0	6
Multiform	4	8	*10*	4	0	0
Paired	2	3	5	*2*	0	0
R-on T	1	0	0	0	*0*	0
VT	0	0	2	0	0	*0*

showed the R on T phenomenon and in 3% runs of VT were recorded One year after the AMI the overall VEB incidence in 122 survivors without reinfarction had increased insignificantly from 71% to 78% The distribution was now 31% infrequent uniform 30% multiform 11% paired and VT 6% (Table I) The R on T phenomenon was not recorded There were no differences in the distribution or incidence of different VEB forms when comparing day with night registrations The most severe VEB form was recorded during exercise in seven instances 75% of patients on drugs with antiarrhythmic action had VEBs prior to discharge and 84% after one year which is similar to the VEB incidence of 75 and 76% respectively in patients without such therapy Nor were any significant differences found when the occurrence of severe VEBs was compared between patients with and without antiarrhythmic drugs

Table I shows the predischarge–one year post AMI VEB course demonstrating a shift towards the more severe forms ($p<0.05$) Accordingly the unaltered benign group amounted to 47 patients (39%) unaltered severe to 23 (19%) impaired to 34 (28%) and 18 (15%) improved

Prognostic weight of VEBs

Ventricular arrhythmias in relation to major cardiac events during the first year are given in Table II Two patients were excluded from the follow up due to death from some other cause than IHD No or uniform VEBs prior to discharge were recorded in 101 patients of whom 4% died suddenly and 11% suffered reinfarction i e 15% major cardiac events In contrast among those with severe VEBs ($n=57$) major cardiac events were recorded in 28% (12% sudden death and 16% reinfarction) This difference in major cardiac events is significant ($p<0.05$)

During the second year of follow up eight of the

Table II *Number of patients with VEBs prior to discharge in relation to major cardiac events during the first year of follow up in 158 patients*

Follow up		Type of VEBs 3 weeks after AMI							
		Benign			Severe				
		None	Uni form	Total	Multi form	Paired	R-on T	VT	Total
Alive not reinfarcted	127	36	50	86	26	12	1	2	41
Major cardiac event									
Sudden death	11	1	3	4	4	0	1	2	7
Reinfarction	20	3	8	11	5	3	1	0	9
Total	158	40	61	101	35	15	3	4	57

No of patients with different types of VEBs in relation to sudden death and reinfarction during the first year of follow up $p<0.05$ for severe VEBs between patients developing/not developing major cardiac events as well as between patients later dying suddenly and survivors without reinfarction Two patients have been excluded because of death not attributed to IHD

for six hour ECG recordings identical with those prior to discharge Two years after the AMI the patients were checked for survival and reinfarction Necessary records were obtained by using the Swedish national or parish registration systems as well as direct contact with the patients themselves

Recording technique

The registrations were performed by telemetry to an inkjet writer (Elema Schonander) run at a paper speed of 10 mm/sec and covered 3 daytime hours during which the patients moved about had lunch and also performed a light exercise The exercise was surveyed by a physio therapist and consisted of climbing stairs until either breathlessness or angina occurred or the pulse rose to 120/min Three hours were registered at night For 20 other patients this procedure was extended to a full day and night recording to evaluate the 6-hour detection rate as compared to 24-hour recordings

Definitions

VEBs were defined as premature QRS complexes with a duration of >0 10 sec with a configuration different from the regular QRS complex and not preceded by a P wave They were conventionally classified (11) in order of severity as infrequent uniform more than 5 uniform/min multiform paired showing the R on T phenomenon or ventricular tachycardias (VT) The R on T phenomenon was defined as complexes with QR /QT<1 and VT as three or more consecutive beats at a higher rate than 100/min Each patient was classified under the most severe VEB form only Patients with more than 5 uniform VEBs/min always had multiform or more severe forms and were graded accordingly After follow up of 100 patients for one year the prognostic value of the different arrhythmias was evaluated *Benign arrhythmias* were on this basis considered present if the registration contained r no or uniform VEBs only the remainder being d as *severe arrhythmias* (20)

course

he patients were divided into four VEB course groups according to the results of the registrations prior to discharge and one year after the index AMI (*a*) Those who at both investigations showed benign arrhythmias=unaltered benign (*b*) Those who at both investigations showed severe arrhythmias=unaltered severe (*c*) Those who prior to discharge only had uniform or no VEBs and one year after the AMI had severe VEBs=impaired (*d*) Those who prior to discharge showed severe VEBs and one year later only had none or uniform VEBs=improved

Reinfarction

Patients were considered to have suffered reinfarction if readmitted because of suspected infarction and fulfilling the criteria of an AMI Patients who died outside hospital with a history of central chest pain lasting for more than two hours and in whom autopsy showed recent myocardial infarction were also included in this group

Sudden death

Patients who according to evidence had died within two hours of onset of symptoms were grouped under this heading Two patients who owing to external circumstances could be resuscitated were also included in this group

Major cardiac events

Sudden death as well as reinfarction were grouped under this heading

Statistical methods

Differences between relative numbers were tested by the χ^2 test and in the case of small absolute numbers Fisher s test The sign test was used for testing intraindividual changes in type of VEBs

Patients

One hundred and twenty seven men mean age 58 years (range 44–65) and 33 women mean age 58 7 years (range 45–65) entered the study During the first year of follow up nine patients died suddenly and another two suffered circulatory standstill but could be resuscitated Twenty patients suffered reinfarction which in five proved fatal Of the patients dying suddenly all but one had symptoms lasting for less than 5 min Autopsy was performed in five of these and revealed clear evidence of a new myocardial event in three Seven patients could not be reinvestigated two of these had died of non cardiovascular causes uremia and diabetes three were alive but had moved abroad one was alive but could not be reached and one refused to participate A total of 122 patients could therefor be reinvestigated after one year

Treatment

During the follow up year conventional therapy was given including cardiac glycosides diuretics and antihypertensive agents Angina pectoris was treated with nitroglycerine and if not adequately controlled reinforced by β receptor blocking agents The arrhythmias detected by the six hour recording were not included in the patient s medical notes and did not constitute a basis for therapy However if the patient developed symptoms judged to be due to an arrhythmia and confirmed by ECG strips or exercise tests antiarrhythmic therapy was not withheld A detailed account of the therapy used in each patient is beyond the scope of this publication but one year after the AMI the proportion of patients receiving cardiac glycosides was 53% and diuretics 60% the number of patients treated for left heart failure amounting to 71% Drugs with antiarrhythmic action were given to 37 patients (26%) Of these one was on quinidine four on β receptor blocking agents one on phenytoin three on verapamil for arrhythmias Beta receptor blocking agents for angina pectoris or hypertension were given to 19 patients phenytoin for epilepsy to two orphenadrine chloride for parkinsonism to one and dipyridamole for angina pectoris to one

RESULTS

Occurrence of VEBs

VEBs were recorded in 75% of the 160 patients prior to discharge In 39% they were infrequent uniform only in 22% multiform in 10% paired 2%

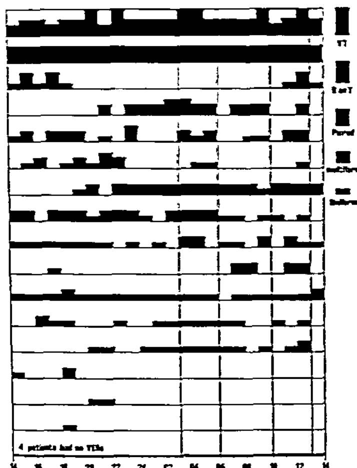


Fig 2 Yield of 6 hours of telemetry rec... ing in comparison to 24 hours in 20 patien...

tic value These relationships when studied in reverse showed that patients who died suddenly and those who reinfarcted did not differ in ventricular irritability incidence of diabetes mellitus or cardiomegaly These findings were also underlined by the fact that three of the five autopsied sudden death patients contrary to the assumed duration of terminal events showed evidence of a ne... myocardial event (19) It thus appears that the... findings of overlap between the two forms of maj... complications of the disease together with the po... results of antiarrhythmic therapy have made th...

Table IV *Occurrence of major cardiac events during the second year of follow-up in relation to arrhythm... profile*

Major cardiac event	VEB profile Unaltered benign ($n=47$)	Improved ($n=18$)	Impaired ($n=34$)	Unaltered severe ($n=23$)
Reinfarction	2	0	2	4
Sudden death	0	1	2	2
Total	2	1	4	6
%	4 3	5 6	11 8	26 1

$p<0.05$

therapeutic standpoint in the post AMI phase even more complex

VEBs registered prior to discharge carried prognostic weight for two years of follow up but not for the second postinfarction year per se for this period however the one year registration provided prognostic information and patients who retained a high degree of ventricular irritability continued to do badly The importance of VEBs as a prognostic indicator thus appears to decrease as time passes as has also been pointed out by Hinkle et al (8) and Moss et al (14)

To what degree therapy has interfered with what might be called the natural history of post AMI ventricular arrhythmias is not known A reduction in incidence or severity of VEBs may well have occurred even though there were no differences between patients treated with digitalis or diuretics for heart failure or β blockers for angina pectoris or hypertension and patients without such therapy

Our registration technique which involved three hours during the day and three hours during the night may be criticized It is not known what length of recordings and during which activity recordings should be performed to provide most useful clinical information (22) Our studies have failed to give a greater prognostic significance for e g VEBs registered at night as compared to those registered uring the day From a purely quantitative view ur registrations as compared to 24 hour regis tions will give a 25% yield However as shown Lown and Wolf (12) the increase in arrhythmic detection follows an exponential curve so that about half of those exhibiting VEBs are detected during the first hour There are two different purposes for a long term registration 1) To correctly describe an individual patient s VEB profile For practical reasons 24 hours is generally considered adequate although the ultimate ideal is the patient s entire life until the final arrhythmia In this study the detection of correct grade of arrhythmias was rather low but the accuracy at grading into correct group benign or severe was high 85% 2) To use a registration which gives prognostic information and in this respect 6-hour registrations were clearly of use Furthermore identical results were obtained in a consecutive series of 10 patients in whom 6-hour recordings were performed on two consecutive days (20) Also our results regarding occurrence of ventricular arrhythmias in the post AMI phase seem very similar to those obtained by others in the same type of patients when longer periods of registrations were performed (5 10 13 16 26)

Our present findings would infer that ventricular arrhythmias in the post AMI phase retain their prognostic value for about one year after the acute event If prognostic information for subsequent periods i e the second post AMI year is required a repeat registration appears necessary To what extent antiarrhythmic drugs presently available are indicated for this target group remains to be studied The end points used i e sudden death and reinfarction do of course per se indicate that a pure antiarrhythmic agent is of limited use and the great overlap between the occurrence of ventricular arrhythmias and extensive myocardial damage in our patients also points to the therapeutic complexity involved The failure to modify ventricular irritability by coronary artery surgery reported by Tilkian et al (24) is of interest in this context Thus not surprisingly antiarrhythmic drugs have so far failed to provide convincingly favourable results (2) In contrast β blocking agents have provided some positive results both regarding the prevention of sudden deaths and reinfarction Here, too further studies are required to identify the most suitable group of patients for such intervention

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Lipoprotein Metabolism in Patients with Chronic Uremia

Effect of Hemodialysis on Serum Lipoproteins and Postheparin Plasma Triglyceride Lipases

Jussi K. Huttunen [1] Amos Pasternack Tuomo Vanttinen [2]
Christian Ehnholm and Esko A. Nikkilä

From Third and Fourth Departments of Medicine the Department of Serology and Bacteriology University of Helsinki Helsinki and the Department of Clinical Sciences University of Tampere Tampere Finland

ABSTRACT Lipoprotein metabolism has been studied in six patients with chronic uremia before and during maintenance hemodialysis Special attention was paid to experimental conditions to control the effect of intermittent heparin administration on various parameters of lipid metabolism The concentration of VLDL was elevated in five of the six patients before dialysis During hemodialysis the concentration decreased in these five patients but increased in the patient with an initially normal VLDL level The concentration of HDL cholesterol was abnormally low before dialysis and increased in all patients after the beginning of dialysis No consistent changes were observed in the concentration of LDL The cholesterol/triglyceride ratio of LDL was significantly lower in uremic patients than in controls both before and during dialysis The activity of postheparin plasma hepatic lipase was markedly decreased in uremic patients both before and during dialysis as compared to age matched controls The activity of postheparin plasma lipoprotein lipase was lower in uremic patients than in controls both before and after the initiation of dialysis but the difference was statistically significant only before the beginning of the treatment Hemodialysis did not affect the activity of postheparin plasma lipoprotein lipase or hepatic lipase The removal constant of i v administered lipid emulsion (Intralipid®) decreased in four patients and increased in one patient after the beginning of dialysis Intermittent heparin administration at two-day intervals in three healthy volunteers did not influence the activity of postheparin plasma lipases The activity of both enzymes decreased during a long term heparin infusion in three patients with deep vein thrombosis After termination of the heparin treatment the activity of both enzymes gradually returned to the pretreatment level

The high incidence of cardiovascular disease in uremic patients (6 23) has stimulated the study of lipoprotein abnormalities associated with renal failure In contrast to the common occurrence of hypercholesterolemia (or mixed lipemia) in nephrotic syndrome and after successful renal transplantation (12) chronic renal failure is characterized mainly by triglyceride elevation (5 12 19 28) Lipoprotein analysis in uremic subjects has usually revealed an increase in very low density lipoproteins (VLDL) and a decrease in high density lipoproteins (HDL) but a normal concentration of low density lipoproteins (LDL) (4 10 19 28 31)

Hemodialysis is known to relieve many of the metabolic abnormalities associated with chronic renal failure (12) However maintenance dialysis has little effect on serum lipids and may even aggravate uremic hypertriglyceridemia under some conditions (4 14 18 24 29 32) These observations have not been adequately explained although it has been suggested that chronic heparin administration changes in nutrition or other factors might counteract the effect of dialysis treatment on serum lipids

We have examined the lipid metabolism in six subjects with end stage uremia before and after initiation of maintenance dialysis Special attention has been paid to the experimental conditions to control the effect of intermittent heparin administration on the various parameters of lipid metabolism

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Present addresses [1]Department of Medicine University of Kuopio Finland [2]Department of Medicine University of Tampere Finland

Abbreviations VLDL=very low density lipoprotein LDL=low density lipoprotein HDL=high density lipoprotein FFA=free fatty acid

Table I *Serum concentrations of total VLDL LDL and HDL cholesterol (Chol) and triglyceride (TG) (mmol/l) in subjects with chronic uremia before and during treatment with regular hemodialysis*

	Total		VLDL		LDL		HDL	
	Chol	*TG*	*Chol*	*TG*	*Chol*	*TG*	*Chol*	*TG*
Uremic patients (n=6)								
Before dialysis	6.4±0.3	2.5±0.3[a]	0.9±0.1[a]	1.5±0.2[a]	4.4±0.2	0.8±0.1	1.0±0.1[a]	0.1±0.02
During dialysis	6.3±0.7	2.1±0.3[a]	1.0±0.2	1.2±0.2	3.8±0.8	0.7±0.1	1.5±0.1[b]	0.2±0.02
Control subjects (n=18)	6.2±0.8	1.2±0.1	0.5±0.1	0.7±0.1	4.4±0.2	0.4±0.1	1.4±0.1	0.2±0.06

[a] Difference from control values statistically significant ($p<0.01$)
[b] Difference between values before and during dialysis statistically significant ($p<0.01$)

PATIENTS AND METHODS

Uremic patients Six male patients with end stage uremia who were expected to need regular hemodialysis within six months participated in the study The age of the patients ranged from 31 to 57 years (mean 50) The histological diagnosis verified by renal biopsy was chronic glomerulonephritis in five and chronic interstitial nephritis in one The average duration of renal disease was 12 years (range 5–19) None of the patients had nephrotic syndrome or proteinuria exceeding 3 g/day at the time of the studies The serum albumin concentration ranged from 2 9 to 4 0 g/l before and from 3 0 to 3 6 g/l during regular hemodialysis

The patients consumed a diet which provided 2 000–2 200 kcal/day and contained approximately 40% of the total calories as fat and 50% as carbohydrate All patients received furosemide aluminium hydroxide calcium carbonate or sodium bicarbonate and vitamins as part of the treatment in the predialysis period In addition arious combinations of α methyldopa hydralazine and dine were given for hypertension During regular alysis furosemide treatment was interrupted in patients and the dose reduced in two Except for sion of sodium bicarbonate the medication remained erwise constant

The patients were dialyzed 4–5 hours daily with a disposable parallel flow dialyzer (18 m²/week) The dialysis fluid contained acetate (35 0 mmol/l) but no glucose The mean creatinine concentration during the predialysis experiments was 1 130 μmol/l (range 715–1 870) During the dialysis period the mean creatinine concentration decreased from 1 280 to 780 μmol/l Changes in body weight amounted to less than 3 kg during the studies in all patients Informed consent was obtained from all participants before the beginning of the experiments

Non uremic subjects The effect of chronic heparin administration on postheparin plasma lipases and on serum lipids was studied in separate experiments in three female patients (without renal disease) receiving i v heparin (200–300 mg/day) as a continuous infusion for treatment of acute deep vein thrombosis The effect of heparin bolus administration at 48 hour intervals (1 mg/kg b wt) was examined in three healthy volunteers without disturbances in lipid metabolism

The control subjects were healthy volunteers with normal weight and normal serum lipid levels (cholesterol 4 5–7 5 triglyceride 0 40–1 65 mmol/l)

Experimental design The uremic patients were hospitalized at least two days before the metabolic studies both in the predialysis period and during maintenance hemodialysis The studies in the predialysis period were performed at 8 a m 40 hours after a 5 hour heparin infusion (35 mg of heparin followed by a continuous infusion of 10 mg/hour) given to simulate the conditions during hemodialysis

The studies in the hemodialysis period were performed 2–4 months after the first hemodialysis The experiments were carried out at 8 a m 40 hours after the previous dialysis and immediately before the next treatment All studies were preceded by an overnight fast

Samples for lipoprotein fractionation were withdrawn in the predialysis period both before heparin infusion and on the morning of the metabolic studies (i e 40 hours after heparin infusion) During hemodialysis the samples were always taken in the morning before the next hemodialysis (i e 40 hours after the end of the preceding dialysis)

Assay of postheparin plasma triglyceride lipase activities The activities of postheparin plasma lipoprotein lipase and hepatic lipase were measured in uremic patients during a 3 hour heparin infusion A priming dose of 1 mg/kg was followed 15 min later by i v infusion of 0 5 mg/kg/hour in 0 9% saline Venous blood samples were withdrawn for the determination of lipase activities 5 35 60 and 180 min after heparin injection In normal subjects the lipase activities were measured 15 min after injection of heparin (1 mg/kg b wt) as described elsewhere (17)

A modified heparin test was used for non uremic patients treated with continuous heparin infusion for deep vein thrombosis To avoid undue bleeding risk the dose of heparin was calculated according to whole blood clotting time measured immediately before the test With clotting time less than 10 min (normal 4–8 min) heparin was given in a dose of 0 4 mg/kg b wt The dose was reduced to 0 3 0 2 and 0 1 mg/kg when the clotting time was 11–19 20–25 and 26–30 min respectively With clotting times exceeding 30 min the rate of heparin infusion was slowed and the test was repeated one hour later In heparin tests carried out before the start of heparin infusion and after the end of the treatment the heparin dose was 1 0 mg/kg b wt (17) The samples for the measurement of plasma lipase activities were withdrawn 15 min after heparin injection

Postheparin plasma lipoprotein lipase and hepatic lipase activities were measured using hepatic lipase antiserum (17) The measurement of lipoprotein lipase activity is

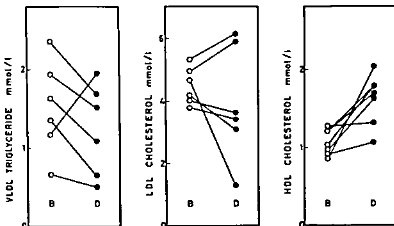


Fig 1 Serum concentrations of VLDL triglyceride LDL cholesterol and HDL cholesterol in six patients with end stage uremia before (*B*) and during (*D*) treatment with regular hemodialysis Means± S E M are given in Table I

based on the inactivation of hepatic lipase by a specific antiserum with subsequent determination of the remaining lipase activity under optimal conditions for lipoprotein lipase (0 1 M NaCl serum activator present) In the assay of hepatic lipase the activity of lipoprotein lipase is inhibited by omitting serum activator and by adding NaCl (final concentration 1 0 M) All assays were carried out in duplicate Each series included two blanks containing saline instead of postheparin plasma and two reference standards taken from two normal subjects and stored frozen at −20°C in small aliquots The lipase activities of each series were corrected if necessary for the mean deviation of the two reference plasma samples from their long term average It has earlier been shown that the enzyme is stable for at least 6 months when stored at −20°C (17)

In separate experiments it was demonstrated that uremic plasma does not interfere with the determination of postheparin plasma lipases in concentrations used in the assay procedure It has earlier been shown that VLDL (VLDL triglyceride less than 10 mmol/l) and LDL (LDL cholesterol less than 15 mmol/l) do not interfere with the assays (17)

Intravenous fat tolerance test was slightly modified from the procedure described by Carlson and Rossner (7) After an overnight fast a bolus of 20% Intralipid was injected i v within 2–4 min The dose was 1 ml/kg ideal b wt plus 0 5 ml/actual−ideal b wt Blood samples were withdrawn immediately before and 2 5 15 20 25 30 50 and 60 min after the injection Plasma was separated and the concentration of exogenous fat particles was determined by nephelometry The concentration was plotted against time on a semi log scale and half life obtained from the linear slope The fractional constant K was calculated from the formula $K = 0\,693/T_{\frac{1}{2}}$

Intravenous glucose tolerance was measured after i v administration of 25 g glucose Blood samples were withdrawn for glucose and insulin assays before and 2 5 10 20 30 40 and 60 min after glucose injection The fractional rate constant was calculated as described above

Laboratory methods Lipoprotein fractionation was carried out using the ultracentrifuge method as described by Havel et al (15) Cholesterol was measured according to Huang et al (16) Triglyceride was determined in a Technicon AutoAnalyzer (Technicon Instruments Tarrytown NY) (20) Plasma insulin was assayed with the method described by Wide (33)

Statistical methods Mean S E M S D linear regression and correlation were calculated with an Olivetti desk computer Mean values were compared with a two-tailed t test

RESULTS

Lipoporotein levels The concentrations of total serum cholesterol and triglyceride and their distribution into the individual lipoprotein fractions in uremic patients and in age matched controls are shown in Table I and Fig I Total serum triglycerides were elevated in all but one patient before dialysis ($p<0\,01$) The concentration decreased during dialysis in five patients and increased in one ($p>0\,1$ NS) The total cholesterol concentration was normal (5 subjects) or slightly elevated (1 subject) No consistent change in total cholesterol was observed during hemodialysis

The change in the concentration of VLDL triglyceride during dialysis parallelled that of total serum triglyceride concentration in all six patients (Fig 1) The effect of hemodialysis on LDL cholesterol and triglyceride was varying (Fig 1) a decrease being observed in four patients and an increase in the other two The concentration of HDL cholesterol was significantly decreased ($p<0\,01$) in uremic subjects before the treatment and increased in all patients during dialysis ($p<0\,01$) (Fig 1 and Table I)

The cholesterol/triglyceride ratio of LDL was significantly lower in uremic patients than in

Table 1 *Serum concentrations of total VLDL LDL and HDL cholesterol (Chol) and triglyceride (TG) (mmol/l) in subjects with chronic uremia before and during treatment with regular hemodialysis*

	Total		VLDL		LDL		HDL	
	Chol	TG	Chol	TG	Chol	TG	Chol	TG
Uremic patients (n =6)								
Before dialysis	6 4±0 3	2 5±0 3[a]	0 9±0 1[a]	1 5±0 2[a]	4 4±0 2	0 8±0 1	1 0±0 1[a]	0 1±0 02
During dialysis	6 3±0 7	2 1±0 3[b]	1 0±0 2	1 2±0 2	3 8±0 8	0 7±0 1	1 5±0 1[b]	0 2±0 07
Control subjects (n=18)	6 2±0 8	1 2±0 1	0 5±0 1	0 7±0 1	4 4±0 2	0 4±0 1	1 4±0 1	0 2±0 06

[a] Difference from control values statistically significant (p<0 01)
[b] Difference between values before and during dialysis statistically significant (p<0 01)

PATIENTS AND METHODS

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The patients consumed a diet which provided 2000–2200 kcal/day and contained approximately 40% of the total calories as fat and 50% as carbohydrate All patients received furosemide aluminium hydroxide calcium carbonate or sodium bicarbonate and vitamins as part of the treatment in the predialysis period In addition various combinations of α methyldopa hydralazine and ˜ idine were given for hypertension During regular alysis furosemide treatment was interrupted in patients and the dose reduced in two Except for ion of sodium bicarbonate the medication remained se constant

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Postheparin plasma lipoprotein lipase and hepatic lipase activities were measured using hepatic lipase antiserum (17) The measurement of lipoprotein lipase activity is

To study the possibility that heparin administered during hemodialysis might influence the activity of postheparin plasma lipoprotein or hepatic lipase we repeated the heparin test (1 mg heparin/kg b wt i e 60–80 mg) in three healthy volunteers four times at two-day intervals The level of the postheparin plasma lipase remained constant during the experiment

A distinct decrease in the activity of both enzymes was observed when the heparin test was carried out at similar intervals during a long term heparin infusion (Fig 5) The activity of lipoprotein lipase fell in two days to less than 20% of the original level and remained low during the rest of the infusion A similar though less pronounced decrease was observed in the activity of hepatic lipase After the termination of the heparin treatment the activity of both enzymes gradually returned to the pretreatment level In one patient the triglyceride concentration increased during heparin infusion and fell again after the end of the treatment No consistent changes were observed in serum triglyceride concentration in two other patients

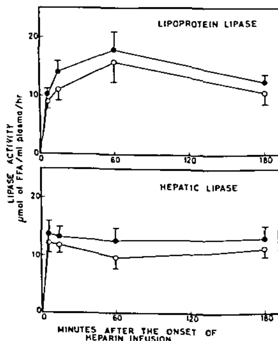


Fig 3 Time course of the activities of hepatic lipase and lipoprotein lipase during a 3 hour heparin infusion in six patients with end stage uremia before (○) and during (●) treatment with regular hemodialysis (mean±S E M)

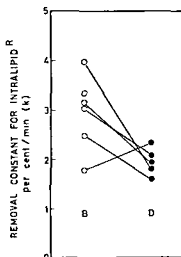


Fig 4 Removal constant for i v administered Intralipid® in subjects with end stage uremia before (*B*) and during (*D*) treatment with regular hemodialysis Means ± S E M are given in the text

DISCUSSION

The lipoprotein pattern observed in the present study in non-dialyzed uremic patients is similar to that described in earlier literature Fasting hypertriglyceridemia has been reported to be common in chronic renal failure and is specifically due to elevated concentration of VLDL (4 10 19 28 31) As noted in the present study the concentration of HDL (measured as HDL cholesterol) is decreased in uremia (4 19 22 28 31) On the other hand the level of LDL generally remains normal As a result of the reciprocal changes in VLDL and HDL total fasting serum cholesterol is also normal except in patients with simultaneous nephrotic syndrome

Contradictory results have been presented concerning the effects of chronic hemodialysis on serum lipids in uremic patients Several investigators have reported that the levels of total and VLDL triglyceride are higher in dialyzed than in non-dialyzed subjects (5 9 24 29) whereas in other studies no differences have been observed in lipoprotein concentrations between the two groups of patients (4 10 14 19) The conflicting data are probably due to several factors operating simultaneously during regular hemodialysis Thus in some patients an increase in dialysis time has lowered serum triglycerides suggesting that ordinary dialysis frequencies are not sufficient for correction of the lipid abnormality (1) Secondly re

Table II *Cholesterol/triglyceride ratio of various lipoprotein fractions (mmol/mmol) in control subjects and in patients with chronic uremia before and after treatment with regular hemodialysis*

	VLDL	LDL	HDL
Uremic patients (n=6)			
Before dialysis	0 70±0 07	5 61±0 34*	6 21±1 08
During dialysis	0 79±0 06	4 87±0 78*	6 49±1 22
Control subjects (n=18)	0 64±0 10	10 8±0 47	8 62±1 11

* Difference from control values statistically significant (p<0 001)

matched controls (Table II) Chronic hemodialysis did not normalize the ratio in any of the patients

Postheparin plasma lipases The activity of post heparin plasma hepatic lipase was markedly de creased in uremic patients both before and after (11 6±1 5 and 13 0±1 9 μmol FFA/ml plasma/hour respectively) the beginning of hemodialysis as com pared to age matched controls (28 0±2 0 μmol FFA/ml plasma/hour p<0 001) (Fig 2) The mean activity of postheparin plasma lipoprotein lipase was significantly lower in uremic patients than in control subjects before the start of dialysis (11 3±1 3 vs 16 9±1 1 μmol FFA/ml plasma/hour) The mean activity during dialysis (14 1±1 3) was not statistically different from the pre dialysis activity or from the activity in the con trols The activities of the two lipases during a 3 hour heparin infusion were not influenced by hemodialysis (Fig 3) No correlation was found between the activities of postheparin plasma lipases and the concentrations of serum cholesterol triglycerides or any of the serum lipoprotein fractions

Removal of Intralipid® Intravenous fat tolerance test was carried out in five patients both before and during hemodialysis and in one patient only before the beginning of the treatment As shown in Fig 4 the rate constant decreased in four patients but increased in one after the initiation of regular dialysis The difference between the mean K values before and during hemodialysis was statistically significant (2 96±0 30 and 1 95±0 13 %/min re spectively p<0 01) No correlation was present be tween the rate constant for Intralipid removal and the activity of postheparin plasma lipases before or during the dialysis period

Glucose tolerance The K value of the i v glucose tolerance increased in all patients during hemodialysis (2 08±0 33 before and 3 36±0 50 %/min during dialysis, p<0 05 paired comparison) Although the mean early (5 min) re sponse of serum immunoreactive insulin to glucose challenge was slightly higher during hemodialysis than before (50±7 before and 65±13 μU/ml dur ing hemodialysis) the change was not statistically significant The late response was similar in both periods

Heparin administration in non uremic patients

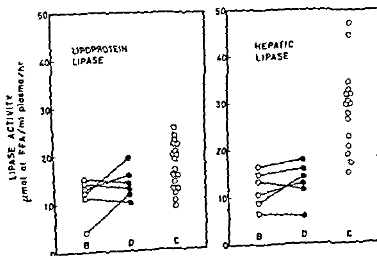
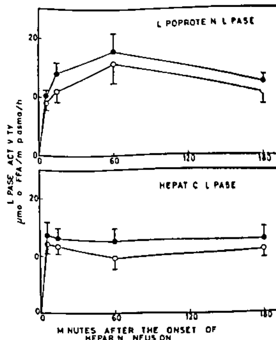


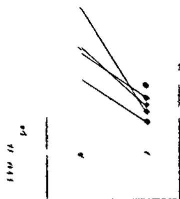
Fig 2 Activities of postheparin plasma lipoprotein lipase and hepatic lipase in six patients with end stage uremia before (*B*) and during (*D*) treatment with regular hemodialysis and in 18 age and sex matched healthy controls (*C*) Means ± S E M are given in the text

To study the poss… … during hemodialysis … pos heparin plasma … we repeated the heparin test (1 … i e 60–80 mg) in three healthy … at two-day intervals. The … plasma l pase remained cons… ment

A dist nct decrease in the … zymes was observed when the … carried out at simil… intervals … heparin infusion (Fig. 5). The … l pase fell in two days to less than 70 … original level and remained low d… the infus on A similar though l ss pr… crease was observed in the a tivity of h… l pase After the termination of the heparin trea ment the activity of both enzymes gradua… re turned to the pretreatment level In one pa… triglyceride concentration increased during heparin infusion and fell again after the end of th treat ment No consistent changes were ob erved in serum triglyceride concentration in two oth r pa t ents



F g 3 T me course of the act v t es of hepat c l pase an l poprote n l pase during a 3 hour heparin infus on in six pat ents w th end stage urem a before (O) and during (●) treatment w th regular hemod alys s (mean+S E M)



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DISCUSSION

Th … pattern observed in th … study … that … been report … m … chronic … level … 33) … in the present study … HDL … creased … 10 … 28 33) … level of LDL generally remain … As a result of the … changes in VLDL … HDL … fasting serum … except in patients with simultane us … syn lrome

C ntrad ct ry results have been presented … cern ng the effects of chronic hem dialysis … serum lipids in uremic patients Several inves tigators have rep rted that the levels of … and VLDL triglyceride are higher in dialyzed th … n ndialyzed subjects (8 9 24 29) … other studies n differences have been … lipoprotein concentrations between the two gr … of patients (4 10 14 19) The … are probably due to sever l … simultaneously during regular he … In s me patients an increase in dialys … were … serum triglycerides … r ry dialysis frequencies are … rectl n of the lipid abn r…

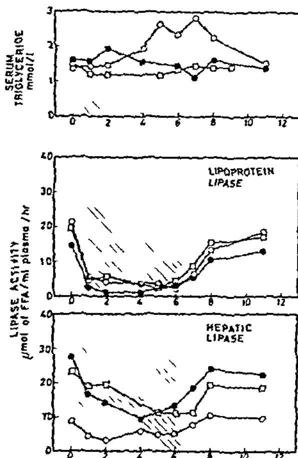


Fig 5 Serum triglyceride concentrations and the activities of postheparin lipoprotein lipase and hepatic lipase in three patients with deep venous thrombosis before, during and after treatment with continuous i v infusion of heparin Treatment with heparin is indicated by the hatched area

peated heparin administration during dialysis may interfere with the removal of triglycerides with subsequent aggravation of hyperlipemia (1 14) Thirdly it has been suggested that incorporation of glucose into the dialysate stimulates the synthesis of VLDL in the liver (2 10) However this is hardly a quantitatively important factor as it has been shown that reduction of dialysate glucose concentration does not improve hypertriglyceridemia (29) Finally it is evident that changes in the diet or in nutritional status after the beginning of dialysis may influence lipoprotein metabolism

The changes in the lipoprotein levels observed in our patients during hemodialysis do not conform in all respects to the pattern reported in the literature Thus total and VLDL triglyceride decreased in five of the six patients studied and an increase in HDL cholesterol took place in all subjects a phenomenon which has not been described before In fact Bagdade and Albers (3) have recently reported that HDL cholesterol is subnormal in both dialyzed and non-dialyzed uremic patients but the two groups were not strictly comparable in their study The reason for the discrepancies between the present and earlier results may lie in the different experimental design In contrast to previous investigations, which have compared separate groups of dialyzed and non-dialyzed subjects we studied the same group of patients before and after the initiation of hemodialysis Moreover all our patients had a severe renal failure at the time of "control" studies a factor which probably facilitated the detection of minor changes induced by dialysis Finally the short interval between the experiments tends to diminish the changes caused by diet and/or nutritional status Thus our results suggest that treatment of chronic renal failure by hemodialysis does not per se aggravate hypertriglyceridemia In contrast hemodialysis seems to partially restore the abnormally low HDL concentration associated with uremia and may decrease the VLDL level at least in some patients

The cholesterol/triglyceride ratio of LDL was significantly lower in uremic patients than in normal controls in keeping with earlier reports (28 31) Furthermore treatment of patients with regular hemodialysis did not normalize the ratio indicating that the biochemical mechanism responsible for the high triglyceride content of LDL is not corrected by hemodialysis Norbeck et al (28) have suggested that the reduction in the LDL cholesterol/triglyceride ratio in uremia is due to the accumulation of the triglyceride rich LDL_1 an "intermediate" lipoprotein in the conversion of VLDL cholsterol rich LDL_2 Against this background it is interesting to note that we found a decrease both in non-dialyzed and in dialyzed patients in the activity of postheparin hepatic lipase an enzyme which might have a role in the conversion of the intermediate lipoprotein to LDL

The mechanism of uremic hyperlipidemia has not yet been fully explained An elevated triglyceride concentration could theoretically be due to increased influx or to impaired removal in the peripheral tissues The evidence for excessive synthesis is mainly circumstantial and is based on the assumption that hypertriglyceridemia is secondary to insulin induced increase in hepatic triglyceride production (5 27) On the other hand several obser

vations suggest that the removal of lipoprotein triglyceride is impaired in renal failure A reduction has been reported in postheparin plasma lipolytic activity both in non-dialyzed and in dialyzed subjects (1 4 5 14 18) Furthermore the triglyceride turnover appears to be decreased in rats with acute experimental uremia (13) and in humans with chronic renal failure (8)

Most of the past reports on postheparin plasma lipolytic activity in uremia have considered this activity to be identical with the adipose tissue lipoprotein lipase the enzyme with a central role in triglyceride removal Recent work has shown however that at least two triglyceride lipases are released into the circulation by heparin (11 17 21) One of these enzymes is identical with adipose tissue lipoprotein lipase whereas the other resembles a triglyceride hydrolase present in heparin perfusates of liver (hepatic lipase) Our results demonstrate for the first time that the activity of both of these enzymes is lowered in uremia The low activity of postheparin plasma lipoprotein lipase is consistent with the reports of low levels of this enzyme in adipose tissue (30) and further supports the existence of a removal defect in uremia However a distinctly more pronounced change was observed in the activity of hepatic lipase and it is probable that the decrease in total postheparin lipolytic activity reported earlier is mainly due to the decrease in the activity of this enzyme The mechanism as well as consequences of the reduction in the activity of hepatic lipase remain unknown

In contrast to Murase et al (25) no inhibition of postheparin plasma lipase activities by uremic plasma was observed in our study The discrepancy is probably due to different assay conditions In our measurement system plasma samples are diluted 1 50 (i e 0 01 ml of plasma to 0 5 ml of the assay mixture) making the interference from different plasma factors less likely

We did not find any consistent alteration in the level of postheparin plasma lipoprotein lipase or hepatic lipase after the beginning of regular dialysis At the same time significant changes were observed in lipoprotein concentrations and in the results of glucose and fat tolerance tests indicating that the treatment influenced other parameters of lipid and carbohydrate metabolism Bagdade (1) has shown that an increase in dialysis frequency from 20 to 40 hours/week sometimes restores pastheparin lipolytic activity to normal level Thus it is possible that our treatment program (15 hours/week) was not sufficient to correct the level of postheparin plasma lipases

The lack of changes in the activity of lipases might also have to do with the repeated administration of heparin in connection with dialysis as suggested by Ibels et al (18) Our control experiments in patients with venous thrombosis indicated that continuous heparin administration can in fact exhaust the tissue store of both lipoprotein lipase and hepatic lipase A similar decrease in the activity of lipoprotein lipase has earlier been demonstrated during short term perfusion of human forearm with heparin (26) Several observations argue however against the possibility that the low postheparin plasma lipase levels during hemodialysis program are due to repeated heparin administration First it was shown that the activity of both enzymes returns to the normal level within 48 hours after the end of chronic heparin infusion i e within the interval between dialyses Furthermore injection of heparin to normal volunteers at 48-hour intervals did not influence the activity of the enzymes It should be emphasized however that with higher dialysis frequencies as in the studies reported by Ibels et al (18) and by Samar et al (31) repeated heparin administration may in fact lead to exhaustion of enzyme stores and thereby interfere with the removal mechanism of triglycerides

Only a few reports have been published so far on the removal rate of i v administered lipid emulsions in uremic subjects According to Ibels et al (18) the rate constant for Intralipid removal is decreased in non-dialyzed patients and in recipients of renal allograft The present study indicates that regular hemodialysis does not improve and may in fact impair fat tolerance in uremia In addition no correlations were found between Intralipid removal rate and the activity of postheparin plasma lipases and the level of serum triglyceride nor between their respective changes after the beginning of hemodialysis Further studies are clearly needed to elucidate the relation between lipoprotein levels postheparin plasma lipase activities and the results of fat tolerance test in dialyzed and non-dialyzed patients with chronic renal failure

ACKNOWLEDGEMENTS

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Familial Lecithin Cholesterol Acyltransferase Deficiency Complicated with Unconjugated Hyperbilirubinemia and Peripheral Neuropathy

The First Reported Cases in the Far East

Aikichi Iwamoto Chikayuki Naito Tamio Teramoto Hirokazu Kato Makoto Kako Toshitsugu Kanya Teruo Shimizu Hiroshi Oka and Toshitsugu Oda

From First Department of Internal Medicine and the Department of Neurology Faculty of Medicine University of Tokyo Tokyo Japan

ABSTRACT Three Japanese patients with lecithin cholesterol acyltransferase (LCAT) deficiency the offspring of a consanguineous marriage are described In addition to the characteristic clinical and laboratory findings of the disease, our patients had hitherto unreported manifestations namely unconjugated hyperbilirubinemia peripheral neuropathy and marked hypocholesterolemia Although the mechanism of the unconjugated hyperbilirubinemia is not clear, the role of impaired hepatic bilirubin uridine diphosphate glucuronyl transferase activity combined with another unknown factor(s) was postulated Non random assortment was observed between LCAT deficiency and haptoglobin types, as previously reported The discovery of Japanese patients with LCAT deficiency indicates that the distribution of this hereditary metabolic disorder is not confined to the Western hemisphere

Familial lecithin cholesterol acyltransferase (LCAT) deficiency is a rare disease with an autosomal recessive mode of inheritance (31 32) Since the first description in 1967 (15 28) 18 patients in nine families have been reported (5 7 13 16 19 27 33 34) Most of them were found in Europe especially in Scandinavia (nine patients) No patient in the Far East has been reported The clinical hallmarks of the disease are corneal opacities with arc formation anemia with target cells proteinuria and foam cells in the bone marrow and glomerular tufts of the kidney

We present here the first three patients with LCAT deficiency in a Japanese family They showed two manifestations not previously reported in association with LCAT deficiency in addition to characteristic findings of the disease All three patients had a history of recurrent jaundice due to unconjugated hyperbilirubinemia and sensory disturbances which were ascribed to peripheral neuropathy

CASE REPORTS

Case 1

A 30-year-old female was referred to the University of Tokyo Hospital because of recurrent jaundice corneal arcus with opacities (Fig 1) and anemia She was born in 1946 the eldest of five children (two daughters and three sons) of Japanese parents who were first cousins Fig 2 shows a summarized pedigree of the family The patient s father was 51 years old and had semilunar corneal arcus without opacities The mother who had been suffering from chronic pancreatitis for many years and precordial discomfort on exertion for one year was 55 years old and had neither corneal arcus nor opacities Neither of the parents had a past history of jaundice

Her corneal arcus had been noticed from childhood In 1962 she was hospitalized for several attacks of tonic cramps At that time the physical findings were unremarkable except for corneal arcus The laboratory tests including electroencephalogram were normal The tonic cramps soon disappeared and she was discharged

She often complained of easy fatigability from childhood Recurrent jaundice was first noticed in childhood and continued until the present admission variable in intensity and irrespective of the menstrual cycle Anemia was first detected in 1969 during her first pregnancy She had three children with normal delivery and growth

On admission her height was 152 cm weight 44 kg BP 104/58 mmHg She was pale and slightly icteric Conspicuous corneal arcus with faint corneal opacities were present Xanthomatous deposits and lymphadenopathy were absent The tonsils and thyroid gland were not enlarged The chest was unremarkable The liver and spleen were not felt Both kidneys were palpable with no noticeable abnormalities Neurologic examination revealed somewhat thickened peroneal and ulnar nerves Pain

Table II *Percentage distribution of cholesteryl ester fatty acids*

	Fatty acid							
	14 0	16 0	16 1	18 0	18 1	18 2	20 0	20 4
Normal subject	2 0	25 8	1 7	5 6	21 3	38 1	1 5	4 0
Case 1	5 7	42 8	0 5	6 1	24 6	20 3		
Case 2	5 8	41 8	2 6	6 8	26 6	16 4		
Case 3	3 9	27 3	2 8	7 1	39 5	19 4		

He was referred to the University of Tokyo Hospital for further studies in 1976

He was 172 cm tall and weighed 64 kg BP 148/68 mmHg He was pale but not icteric There was slight ankle edema Conspicuous corneal arcus with opacities were present He had clinodactyly of the right little finger Other physical findings were unremarkable except for neurologic examination which revealed pupillary inequality (left larger than the right) and impairment of pain over fingers and toes

Siblings

The other two siblings were healthy and had neither corneal arcus nor a past history of jaundice The second sister aged 23 had clinodactyly of the right little finger and questionable impairment of touch and pain in glove and stocking distribution while the third brother aged 24 was normal neurologically

METHODS

Lipids were extracted from plasma or serum and red cells and purified by the method of Folch et al (9) The concentration of serum total cholesterol was determined by the method of Zak and Henly with a slight modification (35) Unesterified cholesterol was determined after digitonin precipitation (30) Lipid phosphorus was determined by the method of Chen et al (6) and phospholipid was calculated as lipid phosphorus×25 Serum triglycerides were determined by the method of Van Handel (20)

Gas chromatographic analysis of fatty acids of the cholesteryl esters was performed following the method of Bowyer et al (4) and Horning et al (23 25)

The main lipoprotein pattern was studied by both agarose gel (26) and polyacrylamide gel electrophoresis (Canalco QDL kit 5635 Fisher Lane Rockville Md 20852 USA) Preparative ultracentrifugation of the patients plasma lipoproteins was performed by the method of Havel et al (21) Each lipoprotein subfraction was analyzed electron microscopically (10)

Plasma LCAT activity was determined by the method of Glomset and Wright (17)

The measurement of the erythrocyte life span and the iron metabolism was performed using ^{51}Cr and ^{59}Fe respectively

Hepatic bilirubin uridine-diphosphate glucuronyl transferase (UDPG T) activity was determined by the method of Black and Billing (3) in needle biopsy liver specimens

Table III *Laboratory values in 1976*

RBC=red blood cells WBC=white blood cells TIBC=total iron binding capacity

	Case 1	Case 2	Case 3	Normal subject
Hb (g/100 ml)	*10 1*	*11 5*	*8 2*	
RBC×10⁴/μl	319	338	273	
Hematocrit (%)	33 1	37 1	26 3	
WBC/μl	3 000	3 600	5 400	
Platelets×10⁴/μl	16 4	20 8	25 5	
Reticulocytes/1 000 RBC	10–54	12–33	9–42	
Prothrombin time (% of normal)	95	100	97 5	
Plasma iron (μg/100 ml)	209	–	65	
TIBC (μg/100 ml)	232	–	144	
Haptoglobin (mg/100 ml)	138	432	428	
Cholesterol of RBC (mg/100 ml of packed RBC)	166	123	153	113
Phospholipids of RBC (mg/100 ml of packed RBC)	359	395	257	346
RBC life span ($T_{\frac{1}{2}}$ d)	28	–	17	
Plasma iron disappearance ($T_{\frac{1}{2}}$ h)	1 72	–	1 67	
RBC utilization (%)	100	–	100	
Plasma iron turnover (mg/kg/d)	0 61	–	0 35	
RBC iron turnover (mg/kg/d)	0 61	–	0 35	

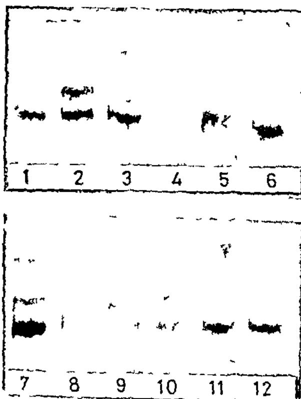


Fig 3 Agarose gel electrophoretograms of lipoproteins of sera of the family members *1*=Serum of a patient with primary biliary cirrhosis *2*=father *3*=mother *4*=case 1 *5*=case 2 *8*=case 3 *9*=third son *10*=second daughter *6 7 11 12*=normal subjects Bands of the α position of the three patients are mainly yellow bands of bilirubin

obtained when patients were on their usual diet without medication

In order to study the effects of fasting on serum bilirubin all three patients were placed on a low fat fasting diet 400 kcal/day

Biopsy specimens of the liver kidney bone marrow sural nerve and subcutaneous nodules were examined histologically

RESULTS

Serum lipids

Values of the major lipid fractions of the family members in the fasting state (14–16 hours fast) during usual diets are given in Table I

The most conspicuous findings were a decrease in esterified to total cholesterol ratio in the three patients and marked hypocholesterolemia in case 3 Case 1 had low total phospholipids and normal triglycerides Case 2 had low total cholesterol normal total phospholipids and elevated triglycerides Case 3 had normal total cholesterol elevated total phospholipid and triglyceride values

Both parents and two siblings—the third son and the second daughter—had normal esterified to total cholesterol ratio

Fatty acids of serum cholesteryl esters of the three patients were mainly mono-unsaturated and saturated (Table II)

Plasma LCAT activity

The values of plasma LCAT activity of the patients are given in Table I expressed as a percentage of the control value (normal adults 21 5±1 6 (S D) μg/ml/hour) LCAT activity was below 10% of the control value in all three patients

Plasma lipoprotein studies

Agarose gel electrophoresis (Fig 3) gave no α lipoprotein band neither could the pre β and β bands be separated in the sera of the three patients Polyacrylamide gel electrophoresis using Canalco kit (Fig 4) showed a decreased α lipoprotein band in all three patients The pre β band was increased in cases 2 and 3 but decreased in case 1 Several abnormal bands were detected between α and β position in all three patients

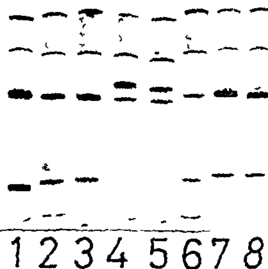
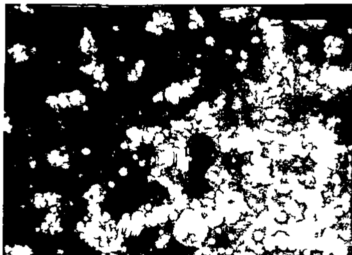


Fig 4 Polyacrylamide gel electrophoretograms of lipoproteins of sera of the family members *1*=Serum of a normal subject *2*=second daughter *3*=third son *4*=case 3 *5*=case 2 *6*=case 1 *7*=mother *8*=father Bands of the α position of the three patients are mainly yellow bands of bilirubin



F g 5 Electron m c ograph of h gh den l popro e ns (1 063<d<1 21) of case 1 o la ed by ul acen rifugat on (Magn f a t on×112 500)

F gs 5 and 6 show electron m croscop c f nd ngs of plasma major l poprote n fract ons of case 1 separated by ultracentrifugat on The h gh density l poprote n fraction (1 063<d<1 21) conta ned abnormal l poprote ns w th the appearance of stacked d sks The low dens ty l poprote n fract on (1 019<d<1 063) varied in size and flattened l poproteins suggest ng l poprote n X were ob served

Hematolog cal stud es

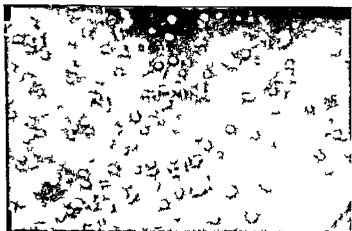
The results of laboratory examinat ons are g ven n Table III All three pat ents had mild to moderate anem a with ncreased number of reticulocytes Peripheral blood smears revealed many target cells (Fig 7) Cholesterol content of erythrocytes was ncreased up to about 1½ t mes the normal per cell n all three pat ents Plasma ron was h gh n case 1 and low n case 3 Haptoglob n was not decreased n any of the three pat ents

The erythrocyte l fe span ($T_{\frac{1}{2}}$) of pat ents 1 and 3 was 28 and 17 days respectively Although plasma and erythrocyte ron turnovers were sl ghtly re duced plasma iron d sappearance t me ($T_{\frac{1}{2}}$) and erythrocyte iron ut lization were normal n ca e 3 wh le ferrokinetic stud es were with n normal l m ts in case 1

Pat ents 1 and 2 were sl ghtly neutropen c The platelet count was normal n all three pat ents

Stud es on unconjugated hyperb l r b nem a

All three pat ents had normal values for conjugated b l rub n but unconjugated bil rub n was elevated in pat ents 1 and 2 to 3 65 and 1 44 mg/100 ml re



F g 6 Electron m c ograph of low dens y l popro e ns (1 019<d<1 063) of case 1 so la ed by ul racentrifuga on (Magn f ca t on×56 250)

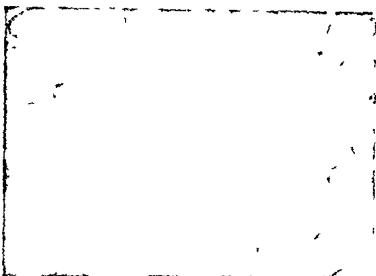


Fig 7 Target cells revealed in the peripheral blood smears (May–Grunwald–Giemsa magnification×1460)

spectively During a three month observation total bilirubin (unconjugated bilirubin is shown in parentheses) in patient 1 fluctuated between 4 20 (3 65) and 1 90 (1 60) mg/100 ml The total bilirubin of patient 2 ranged between 2 88 (2 58) and 1 84 (1 44) mg/100 ml during a two-week observation The total bilirubin of patient 3 remained below 1 mg/100 ml constantly during a three month observation

After two days on a low fat–low calory diet (400 kcal/day) total bilirubin increased from 1 90 to 5 31 g/100 ml in patient 1 (179% rise) and from 0 97 to 39 mg/100 ml (43% rise) in patient 3 while no crease was observed in patient 2 The rise of total ilirubin observed in patients 1 and 3 was mainly due to an increase in unconjugated bilirubin Neither serum haptoglobin nor red cell count was reduced during these experimental periods

Hepatic bilirubin UDPG T activity was very low compared with control values (two cases with chronic hepatitis) in all three patients 0 29 0 58 and 0 20 mg/g/hour in patients 1 2 and 3 respectively versus 1 07 and 1 35 mg/g/hour in control subjects

Daily administration of 180 mg phenobarbital for two weeks decreased the total bilirubin from 1 96 to 0 63 mg/100 ml in patient 1

Other laboratory studies

Table IV shows the results of routine laboratory tests All conventional liver function tests were normal in all three patients Case 1 had minimal proteinuria and case 2 moderate proteinuria with normal renal function tests while case 3 had massive proteinuria with moderately decreased endogenous creatinine clearance Serum protein and albumin were low and serum uric acid was high in cases 2 and 3 The peroral glucose (100 g) tolerance test was normal in all three patients

X rays of the chest abdomen i v pyelogram i v cholangiogram or oral cholecystogram and electrocardiogram were normal in all three patients

Table IV *Laboratory values in 1976*

SGOT=glutamic oxaloacetic transaminase SGPT=glutamic pyruvic transaminase LDH=lactic dehydrogenase

	Case 1	Case 2	Case 3
Serum protein (g/100 ml)	7 4	6 2	4 3
Serum albumin (g/100 ml)	4 4	3 4	2 1
Total bilirubin (mg/100 ml)	4 20	1 84	0 97
Conjugated	0 55	0 40	0 20
Unconjugated	3 65	1 44	0 77
Alkaline phosphatase (K A unit)	5 0	9 0	5 4
SGOT (K unit)	15	17	22
SGPT (K unit)	7	9	10
LDH (UV unit)	244	212	230
Uric acid (mg/100 ml)	4 37	8 28	9 32
Urea nitrogen (mg/100 ml)	17	13	22
Creatinine (mg/100 ml)	0 59	0 93	1 16
Daily urinary protein (g/d)	0 5	1–3	8–12
Endogenous creatinine clearance (ml/min)	115	82	49



Fig 9 Liver section embedded in epoxy resin *H*=hepatic cells *E*=endothelial cell *D*=Disse s space *S* sinusoid *C* collagen fibrils (Magnification×8395)

Electroencephalogram (EEG) electromyogram (EMG) and motor (MCV) and sensory nerve conduction velocity (SCV) were examined in two patients (nos 1 and 3) EEG showed dysrhythmic α activity with slightly slow waves but no epileptic patterns in case 1 and was normal in case 3 EMG was interpreted as neurogenic in case 1 but was unremarkable in case 3 Although MCV was normal SCV was decreased over distal parts of extremities in both patients (Table V)

Cerebrospinal fluid examined in patients 1 and 3 was normal except for a low protein value in patient 3

Fig 1 shows haptoglobin types of the family members All three patients had the same haptoglobin type which was different from that of other family members without signs of the disease

Histological examinations

Normocellular bone marrow with relative erythroid hyperplasia was observed in all three patients Erythroid cell series myeloid cell series and megakaryocytes were normal morphologically No sea blue histiocyte was detected but a few foamy cells were noted in the bone marrow of case 2 (Fig 8)

The liver biopsy specimens of the three patients showed the same pathological findings in variable degree Light microscopy revealed a partial widening of Disse s space A mild portal fibrosis but no inflammatory cell infiltration was present On electron microscopy Disse s space appeared widened and granular and amorphous deposits were detected Microvilli were depleted and liver cell membrane was partly disrupted A number of large

Table V *Nerve conduction velocity (m/sec)*

MCV motor nerve conduction velocity SCV=sensory nerve conduction velocity

	Case 1	Case 3
MCV		
Right ulnar nerve	61 8	60
Right peroneal nerve	41 3	55 8
SCV		
Right medial nerve		
Middle finger to wrist	36 9	29
Wrist to elbow	59 8	70
Right peroneal nerve		
Instep to ankle	15 4	20
Ankle to knee	32 2	68 8



Fig 10 Foamy cell in the glomerular tufts of case 2 (Periodic acid Schiff magnification×353)

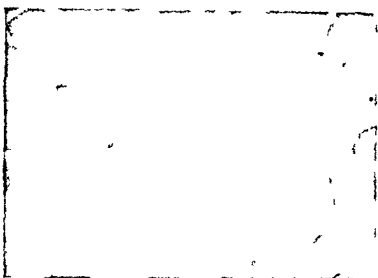


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Serum Aminotransferases after Low-Dose Heparin Treatment

Short Communication

R Olsson B M Korsan Bengtsen K Korsan Bengtsen J Lennartsson and J Waldenstrom

From the Departments of Internal Medicine II and Clinical Chemistry University of Gothenburg Sahlgren s Hospital Gothenburg Sweden

Low-dose heparin treatment has repeatedly been reported to effectively prevent postoperative thrombosis It has therefore also been introduced for thrombosis prophylaxis during the first days after acute myocardial infarction Since Sonnenblick et al (3) have reported elevated aminotransferase levels after moderately high i v heparin doses (10 000 U every 6 hours) to patients with thromboembolic diseases we considered it important to study whether low-dose subcutaneous heparin treatment may also induce elevated aminotransferases which may cause erroneous suspicions of reinfarction or liver disease

PATIENTS METHODS AND RESULTS

Three groups of subjects were studied *Group A* 13 hospitalized patients (mean age 75 years range 62–94) with cerebrovascular accidents at least two weeks previously None had clinical signs of myocardial infarction heart failure thrombophlebitis pulmonary embolism liver or muscle disease or other factors including drugs known to affect serum aminotransferases They were given subcutaneous heparin injections (5 000 U every 8 hours) for 10 days *Group B* 9 healthy medical students (mean age 25 years range 22–47) who were given subcutaneous heparin injections (5000 U every 8 hours) during 20 days The study on this group aimed at testing whether a new analgetic drug nefopam (Acupan® Riker) could be given safely to patients on anticoagulant therapy From the 7th to the 13th day of heparin treatment they therefore also received the test drug *Group C* 15 patients (mean age 57 years range 33–73) with mainly valvular heart disease and atrial fibrillation A similar study was performed with heparin replaced by dicoumarol and with nefopam for 14 days

The activity of serum aminotransferases (aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT)) lactic dehydrogenase (LD) alkaline phosphatases (ALP) bilirubin and in five patients creatine kinase (CK) were analysed at intervals during and after treatment S ALAT S ASAT S LD and S-ALP were determined using the methods recommended by the Scandinavian Committee on Enzymes (1) The upper reference value for both aminotransferases was 0 7 μkat/l and for S-LD 8 μkat/l

In group A four patients showed increases above the upper limit of normal in both aminotransferases (Fig 1) an additional patient had a slight S-ALAT elevation (0 88 μkat/l) on the 8th day In all patients a decrease occurred after discontinuation of treatment starting in two already during treatment S ALAT showed the most pronounced elevations in all patients S-CK was studied in three of the illustrated patients and in two of the patients whose aminotransferases did not change No significant changes were observed S LD rose successively from 6 8 to 10 5 μkat/l in the patient with the most pronounced S ALAT elevation On the 7th day of treatment the rise appeared to be caused by liver isoenzyme whereas on the 3rd day after treatment it appeared to be of myocardial origin None of the other patients showed any significant S LD changes S ALP did not change in any of the patients Eight patients including the four with aminotransferase elevations showed successive decreases in their bilirubin values from a mean of 16 6 to 10 6 μmol/l after 7–8 days of heparin whereas the remaining five patients showed no change Therefore the mean bilirubin concentration in the whole group on day 7–8 (11 1 S D 4 46) was not significantly lower than before the heparin injections (14 8 S D 1 84)

In group B there was a rise of the aminotransferases in all but one subject (Fig 1) The rise was observed after a similar period of treatment as in the above mentioned patients but was usually more pronounced The aminotransferases usually started to decrease again already during heparin treatment

In group C no changes were observed in aminotransferase activity

DISCUSSION

Four of the patients in group A had aminotransferase elevations in contrast to 10 out of 14 in the study of Sonnenblick et al (3) This difference may indicate a dose-dependent reaction However the reaction may also be age-dependent since the mean age of our patients was 75 years against 39 years

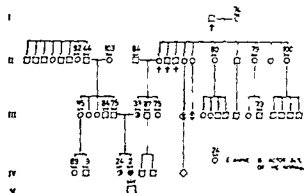


Fig 1 Pedigree IV 4 is the proband

Table II *Female haemophiliacs who might be heterozygotes*

	No of cases	
	Haemophilia A	Haemophilia B
Father affected mother non-carrier	1	1
Father healthy mother carrier	5	3
Father healthy mother non-carrier (spontaneous)	5	1
Total	11	5

A few of the reports were of less recent date and did not give exact factor VIII or IX levels but nevertheless clearly showed that the women had severe haemophilia These cases were included

Of the 32 women 9 were certainly homozygotes as their fathers were affected and their mothers proven or probable carriers (9 16 17 23 33) or mutations that cause testosterone receptor defects Chromosomal aberrations could explain 7 cases 4 of haemophilia A (2 8 20 29) and 3 of haemophilia B (3 18 30)

This leaves 11 cases of female haemophilia A and 5 of female haemophilia B still to be explained These 16 cases fall into three groups (Table II) In first group the father was affected but the showed no evidence of a carrier (11 13) In second group the father was healthy but the mother was a proven or probable carrier (5 7 13 15 19 25 28 32) In the third group the so-called spontaneous cases the father was healthy and there was no evidence that the mother was a carrier (1 4 6 24 27 31) It must be borne in mind however that if the mother s family has not been very thoroughly investigated one cannot be at all certain whether or not she is a carrier It is therefore probable that some of the cases in the third group should have been included in the second Furthermore in two of the cases no cytogenetic data were available

Table I *Factor IX activity and antigen (%) in the proband (IV 4) her mother (III 6) and her son (V 1)*

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ᵃ A low level of factor IX activity in 1960 but a normal level in 1977 She is now postmenopausal Factor IX tends to rise with age

ᵇ One month after delivery A temporary rise in factor IX activity to 6% late in pregnancy

ᶜ At 2 weeks of age

The above mentioned 16 females may be heterozygotes However their very low levels of factor VIII or factor IX and severe symptoms are more compatible with the homozygous expression of haemophilia Homozygosity is theoretically possible if there has been either a mutation in the X-chromosome from the father or the mother or non-disjunctions (e g loss of one X-chromosome in one of the gametes followed by a second postzygotic non-disjunction) Whether these severely affected female haemophiliacs are heterozygotes or not can be determined only by studying their offspring

Our case is the first example of a severely affected female haemophiliac having born a healthy son The proband has haemophilia B of the B variety as her factor IX antigen is just as low as her factor IX activity (22 26) In her boy both factor IX antigen and activity are normal for age His karyotype is normal 46 XY His mother must therefore be heterozygous for the haemophilia gene

The existence of frank haemophilia in such a woman seems to challenge the rules of X linked inheritance The phenomenon can most readily be explained by Lyon s hypothesis of X-chromosome inactivation The problem is that at the probable time of X-chromosome inactivation in man the embryo already consists of some thousands of cells

The probability of the same X chromosome being inactivated in all or nearly all of these cells is very small For example the frequency of homozygous expression of the haemophilia phenotype in a heterozygote would be only 1×10^{-12} if the X chromosome inactivation occurred at a cell number of 40 (14)

Although this is the first report of a proven heterozygote with a homozygous expression of haemophilia some of the other published cases of female haemophilia are probably of the same type If so it would mean that the X chromosome inactivation must occur at an embryonal stage of perhaps not more than 20 cells which seems to invalidate Lyon s hypothesis as an explanation of the observed phenomenon However the only interesting cells in our case are the precursors of liver cells which form factor IX and not the cells of the whole embryo It is quite possible that the hepatocytes of the adult are derived from rather few cells at the time of X inactivation

In female germ cells both X chromosomes are active even if there has been an extreme inactivation of one of the X-chromosomes in somatic cells A woman heterozygous for the haemophilia gene but with a homozygous expression of the disease should have the same chance of getting a healthy boy as a haemophilic one

ACKNOWLEDGEMENT

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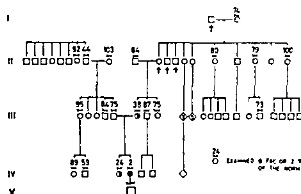


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Bartter's Syndrome without Hyperplasia of the Juxtaglomerular Apparatus, Treated with Indomethacin

H J Kornerup E B Pedersen and V Posborg Petersen

From Department of Medicine C Århus Kommunehospital and Århus University Århus Denmark

ABSTRACT The present report describes a case of potassium wasting nephropathy with the physiological and endocrinological findings that are typical for Bartter s syndrome (BS) However the renal juxtaglomerular apparatus showed no hyperplasia at two renal biopsies two years apart The short term (9 days) effect of indomethacin in combination with spironolactone was a suppression of hyperreninemia and hyperaldosteronism and an increase in vascular sensitivity to angiotensin II associated with potassium and sodium retention Subsequently on indomethacin alone, potassium balance was obtained on a lower level with persistent hypokalemia and persistent renal potassium leakage Hypokalemia persisted during long term (9 months) treatment with indomethacin despite normalization of the activity of the renin–aldosterone system The results indicate that indomethacin as long term treatment may be ineffective in maintaining a normal potassium balance in BS

Bartter et al (1) described in 1962 a new syndrome consisting of hyperplasia of the renal juxtaglomerular apparatus (JGA) hyperreninemia resistance to the pressor effect of angiotensin II hyperaldosteronism and hypokalemic alkalosis due to renal potassium wasting Subsequently several patients with such findings have been reported as cases of Bartter s syndrome (BS) In these previous reports whenever renal biopsy was performed JGA hyperplasia has been emphasized consistently as an obligatory finding for BS (25) Recently it was suggested that an increased renal synthesis of prostaglandins may be of pathophysiological importance in BS as treatment with inhibitors of this synthesis have been reported to induce a reversion of the main features of the syndrome (6 11 18 24)

In the present study we report a case of potassium wasting nephropathy associated with the functional abnormalities of the renin–aldosterone system that are characteristic for BS but without JGA hyperplasia In addition the effect of long term treatment with indomethacin is reported

CASE REPORT

A 41 year-old man was admitted to a neurological ward on Nov 10 1974 because of quadriplegia and paralytic respiratory failure which had developed during the course of a few hours These alarming symptoms had been preceded by varying degrees of muscular stiffness for one month Otherwise he had previously been completely healthy and denied the use of diuretics licorice purgatives or other drugs The preliminary blood analyses disclosed an excessive hypokalemic alkalosis with serum potassium of 0 8 mEq/l and total carbon dioxide in serum of 31 mEq/l The paralyses disappeared completely 6 hours after correction of the hypokalemia He was transferred to our department on Nov 22 1974

Examination revealed a man in good general condition with normal physical findings No edema was present and BP was 110/65 mmHg As illustrated in Fig 1 the predominating feature was excessive renal potassium wasting with urinary potassium excretions above 200 mEq/24 h excreted potassium accounting for 60–70% of filtered potassium A gradual increase in oral potassium accentuated the renal potassium wasting and hypokalemia persisted with serum potassium values of about 2 0–2 5 mEq/l The levels of other serum electrolytes were normal (sodium 138 mEq/l chloride 100 mEq/l calcium 10 2 mg/100 ml and phosphorus 3 2 mg/100 ml) except for serum magnesium which was slightly decreased 1 1 mEq/l The values of plasma renin concentration (PRC) and plasma aldosterone concentration (PAC) were markedly increased on a free diet (Fig 1) Simultaneously the vascular sensitivity to angiotensin II was slightly decreased (12 6 ng/kg/min)

PRC was measured as described by Giese et al (10) normal range 10–79 micro Goldblatt units/ml plasma (μGU/ml) PAC was determined according to the method

Abbreviations BS=Bartter s syndrome JGA=juxtaglomerular apparatus BP=blood pressure PRC=plasma renin concentration PAC=plasma aldosterone concentration μGU=micro Goldblatt unit RBC=red blood cells

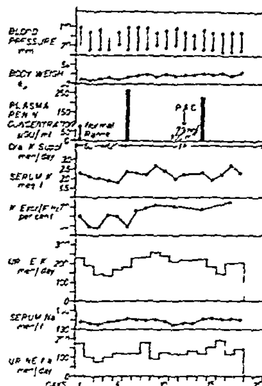


Fig 1 Initial clinical course

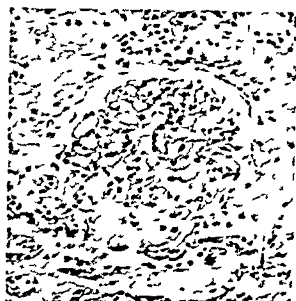


Fig 2 Renal biopsy Dec 2 1974 Photomicrograph of a glomerulus showing the absence of JGA hyperplasia. Hematoxylin-eosin staining ×240 A repeat renal biopsy Sept 23 1976 was identical

of Damkjær Nielsen (20) normal range 4 6–25 9 ng/100 ml Sensitivity to the pressor effect of exogenous angiotensin II (Hypertensin Ciba) was assayed as described by Kaplan and Silah (14) normal range 6 0–11 4 ng/kg min

The levels of other hormones in plasma or urine were plasma epinephrine 0 05 ng ml plasma norepinephrine 0 14 ng/ml plasma cortisol 13 4 μg/100 ml 17 ketosteroids and 17 ketogenic steroids in urine 11 1 and 13 6 mg/24 h respectively

Histology On light microscopy a percutaneous renal biopsy of the left kidney performed on Dec 2 1974 showed normal JGA without hyperplasia of the juxtaglomerular cells (Fig 2) No hyperplasia of the interstitial renomedullary cells was present. The tubular cells were normal without kaliopenic vacuoles No glomerular changes were present Except for slight interstitial fibrosis and arteriolosclerosis findings were negative A new percutaneous renal biopsy of the left kidney performed on Sept 23 1976 was identical with the first one Electron microscopy was normal except for small vacuoles in the luminal part of tubular cells

Kidney function Glomerular filtration rate and renal plasma flow examined by a constant infusion technique using [125]I-iothalamate and [131]I-hippuran as reference substances (21) were normal (117 and 517 ml min respectively) The ability to concentrate and to acidify urine was almost abolished (urinary osmolality 410 mOsm kg after 24 hours thirst urinary pH 6 2 after oral load of ammonium chloride 6 g day for 2 days) Fig 3 demonstrates a normal ability of the kidneys to conserve sodium during oral sodium restriction for 10 days No proteinuria was

Table I *Renin–aldosterone system during changes in sodium and potassium balance*

	PPC (μGU/ml)	PAC (ng 100 ml)	Serum potassium (mEq/l)
Low sodium intake (10 mEq day for 10 days)	215→363	26→59	2 4→2 5
High sodium intake (300 mEq day for 5 days)	275→164	9→18	2 1→2 6
Acute i.v. sodium load (450 mEq/3 000 ml/90 min)	155→84		
Withdrawal of potassium supplement for 7 days	250→477	27→21	2 2→1 7
Oral potassium load (80 mEq 6 h)	370→325	19→122	1 9→5 3

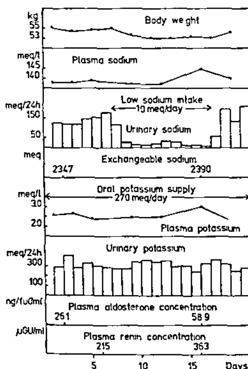


Fig 3 Effect of prolonged sodium restriction

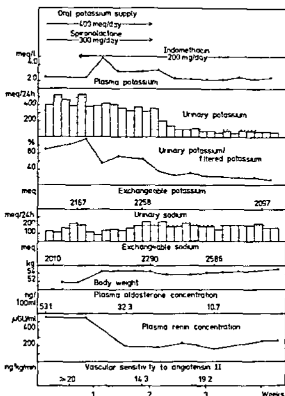


Fig 4 Effect of treatment with indomethacin —=Potassium and sodium intakes

present The morphology of the urinary sediment was normal and urine cultures were negative A pyelogram was normal Glucosuria was absent and urinary excretion of amino acids was within the normal range

Renin–aldosterone system Table I shows the effect of changes in sodium and potassium balance on PRC and PAC The basal PRC was increased 8–10-fold compared with the mean value in normals PRC increased normally during sodium restriction as well as during withdrawal of potassium supplements and decreased normally during sodium loading Yet potassium loading did not suppress the hyperreninemia The basal PAC was slightly increased However potassium loading disclosed an excessive hyperaldosteronism

Potassium cell membrane transport ^{42}K influx studied in lymphocytes using the method of Hansen and Clausen (13) was normal (1 125 ions/binding site/min compared with a control value of 1 277 and the number of binding sites/cell was 52 508 which is normal) Potassium and sodium contents in RBC examined as described by Funder and Wieth (7) were also normal (101 7 and 11 4 mEq/kg RBC respectively)

Effect of triamterene and spironolactone Triamterene in a dosage of 400 mg/day was given from Jan 20 to Nov 10 1975 and spironolactone in a dosage of 400 mg/day from Dec 10 1975 to Sept 30 1976 These agents were not able to prevent renal potassium wasting

Effect of indomethacin Spironolactone (400 mg/day) and an oral potassium supplement (400 mEq/day) were supplemented with indomethacin in a dosage of 200 mg/day on Sept 22 1976 As illustrated in Fig 4 this combination of therapy (2 weeks) induced a decrease in PRC and PAC and an increase in sensitivity to the pressor effect of angiotensin II Simultaneously there was potassium retention associated with a transitory increase in serum potassium and sodium retention Exchangeable potassium and sodium were determined as 24 hour exchangeable ^{42}K and ^{24}Na respectively using a dilution technique according to the principles given by Moore et al (17) After withdrawal of spironolactone and reduction of potassium intake treatment with indomethacin alone was continued On this regimen of indomethacin combined with a reduced but still high potassium intake (100 mEq/day) potassium balance was obtained on a lower level with persistent hypokalemia and persistent renal potassium leakage as indicated by a reduced but still high ratio urinary excreted/glomerular filtered potassium

Indomethacin in a dosage of 150–200 mg/day was given from Sept 22 1976 to June 13 1977 Due to persisting hypokalemia indomethacin was combined with an oral potassium supplement of 120 mEq/day from Oct 28 1976 to May 16 1977 On this therapy PRC was further suppressed and eventually normal (48 µGU/ml) and PAC remained normal (16 7 ng/100 ml) but hypokalemia persisted with serum potassium values of 2 8–3 0 mEq/l In addition the ability to concentrate and to acidify urine improved (maximal urinary osmolality 754 mOsm/kg and minimal urinary pH 5 3)

Family The father had died in 1958 (60 years old) from

ANNOUNCEMENT

International Symposium on Antibiotics and Hospitals sponsored by Societa Italiana di Farmacia Ospedaliera Associazione Microbiologi Ospedalieri Italiani Fondazione Giovanni Lorenzini will be held in Milan Italy Nov 24-25 1978

Further information Organizing Secretaria Fondazione Giovanni Lorenzini Via Monte Napoleone 23 I 20121 Milan Italy

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MODERN MEDICAL HISTORY

The Broad Tapeworm Story

The inhabitants of northern latitudes are preserved from many parasitic diseases causing severe suffering and misery farther south However two macroparasitic diseases have played an important role in the medical history of the Nordic countries echinococcosis and diphyllobothriasis Like all parasitic diseases diphyllobothriasis has a biological and ecological as well as a medical aspect

In 1602 F Platter found that human beings could be infested with two kinds of tapeworm *species prima* or broad tapeworm and *species secunda* or *Taenia* A distinction between *T saginata* and *T solium* was not made until the middle of the 19th century

The broad fish tapeworm *Diphyllobothrium latum* is well known and precisely defined That other tapeworms exist in whose life cycle fishes serve as intermediate hosts is not so generally known among the medical profession Human infection has proved to be possible with some of these species both experimentally and spontaneously This is true in particular for *D dendriticum* whose natural final hosts are gulls *D dendriticum* has a circumpolar somewhat more northerly extension than *D latum* Instances of spontaneous human infection are known from Siberia in any event Other mammals e g dogs may also become infected In the case of human infection with *D dendriticum* it usually does not take more than a few months for the worm to be expelled since man is not its natural final host By contrast a human *D latum* carrier may harbour his tapeworm for years sometimes decades Besides *D latum* and *D dendriticum* a great many species of ***Diphyllobothrium*** have been described and many of them have been reported to cause human infection Authentic cases have been published from Alaska It seems likely that many of the species described as separate are identical with *D dendriticum* but a further analysis of the situation in the arctic regions of Siberia and North America is desirable The lack of reliable taxonomic criteria constitutes a problem however The definition of such criteria is an important task for helminthological research today

The course of human infestation remained a mystery for a long time a *generatio spontanea* was generally believed to occur In 1747 H D Spöring suggested that the broad tapeworm developed in man after the consumption of raw or insufficiently *cooked fish and some later authors expressed similar* thoughts A long time elapsed however before M Braun—in about 1880—succeeded in showing that pike and burbot from Lake Peipus in Estonia harboured larvae of the broad tapeworm plerocercoids with which it was possible to induce infection in humans Later it was found that larvae of *D latum* also occurred in perch and ruff and many salmonids The widespread belief that coregonids (whitefish vendace) also harbour larvae of *D latum* is erroneous though difficult to eradicate

The course of infestation of the fish long remained an unsolved question however It proved impossible to infect fish with eggs or with the coracidia hatched from eggs of *D latum* At last C Janicki and F Rosen two Polish investigators working in Switzerland were able in 1917 to show that the coracidia had first to be taken up by an intermediate host of copepod type in whom they developed to procercoids The life cycle of *D latum* was thus found to be as follows eggs of the parasite present in human or mammal faeces are deposited in water courses where the coracidia are hatched and taken up by copepods (first intermediate host) plankton including infested copepods is consumed by fishes (second intermediate host) in whom the procercoid develops to a plerocercoid It has later been noticed that an infected fish is often swallowed by a larger predatory fish (carrier host) which becomes the definitive source of infection for the final host The worm adapts itself best to man but domes



Felix Platter (1536–1614) Professor of Medicine University of Basle Switzerland Portrait by Hans Bock the Elder Kunstmuseum Basle Switzerland

mals (dogs cats pigs) and wild animals (e g foxes) may also become carriers of the broad tapeworm Research on *Diphyllobothrium* got a strong push forward from V G Gnezdilov s discovery (1957) that the golden hamster could be infected and used as a laboratory animal By contrast culture of *D latum* in vitro has not been successful

Endemic foci of *D latum* develop only under certain favourable conditions Copepods and fishes suited for the role of intermediate host are a prerequisite They require a habitat of fresh or slightly brackish water (maximum salt concentration 0 2–0 4%) with a temperature mostly under 22°C The best biotopes for the various larval stages are relatively shallow lakes or slow flowing rivers with a suitable vegetation Within the Russian endemic region *D latum* has been widely spread in the system of power plant dams water reservoirs and canals constructed during the last few decades Finally the maintenance of the life cycle of *D latum* requires the presence of appropriate final hosts fish-eating mammals and humans with a taste for raw fish and raw hard roe Among the tapeworm-infected Finnish population it has been customary to eat slices of freshly caught fish (peak perch) raw or slightly brined and hard roe of pike and of burbot in particular has been considered a great delicacy It seems likely that dietary habits have been similar in the Russian tapeworm region Raw liver of burbot has also been highly appreciated among the fishermen around Kurish Bay and previously in Switzerland

Epidemiological research on *D latum* is concerned with both the occurrence of plerocercoids in fish and the prevalence of infection in man (examination of faeces for tapeworm eggs)

In Scandinavia Finland is known for its broad tapeworm endemics Before the 1950s about 20% of the total Finnish population was considered to be infected Prevalences of nearly 100% were noted in the lake districts while other parts of the country



Herman Diedrich Spöring (1701–1747) Professor of Medicine University of Åbo/Turku Finland Portrait by G E Schröder State Medical Board Stockholm Sweden

were practically free from the parasite Since then a rapid decrease in the prevalence has occurred mainly as a result of social changes so that today it is only one tenth of what it was some 20–30 years ago The parasite has also been reported to occur in those regions of north Sweden which border on Finland

It would be a great mistake however to believe that the Finnish endemic is unique *D latum* has its widest distribution in certain parts of the Soviet Union although the parasite has previously attracted less attention there than in Finland Estonia the Leningrad region Soviet Karelia the Kola Peninsula the coastal region from Lake Onega to the Pechora River and above all the entire huge Volga Basin have been and apparently still are in part heavily infected The westernmost endemic known of old is found round Kurish Bay To the east the tapeworm reservoir extends as far as the Ob Basin and seems even to include the region of the Yenisey to some extent The Danube Delta and the region of the River Prut are notorious for their endemics The majority of epidemiological reports on *D latum* presented at symposia or published in parasitological journals in the Soviet Union refer to the above mentioned regions Last year a *Diphyllobothrium* bibliography was published in Petrozavodsk by A I Rosenberg It contains 2218 Russian and 971 other references from the years 1552–1972 and yet it is by no means complete Of the Russian papers not less than 1725 were published between 1950 and 1972

In the Soviet Union the chief objects of research on *Diphyllobothrium* have been epidemiology prevention and therapy Ethnological aspects have attracted little interest In Finland it is a well known fact that the East Finnish (Savo-Karelian) dialectal region has been the principal area of distribution of the broad tapeworm while no true endemic has occurred in the West Finnish dialectal region nor among the Swedish speaking population although suitable biotopes have not been missing The reason for this is that the custom of eating raw fish has on the whole been limited to the eastern region Looking at the map of the Soviet Union one is struck by the fact that as far as can be judged by an outsider no *D latum* foci occur in the vast area between the River Volga and the western border of the Union although this area includes both rivers with power plants canals and lake districts It is a tempting conclusion that there must be a



Max Braun (1850–1930) Professor of Zoology University of Dorpat/Tartu Estonia (later Rostock and Konigsberg)

difference in dietary habits between this area and the regions of tapeworm endemics It is moreover striking that populations belonging to the Finno-Ugrian linguistic group have been and still are resident precisely in these regions (Estonia the Leningrad district Karelia the region round the upper and middle course of the Volga the areas inhabited by the Ob Ugrian ethnic groups the areas inhabited by the linguistically related Laps and Samojeds) Languages and customs dietary habits not least often run together Perhaps the custom of consuming raw fish has been an ethnic characteristic of the Finno-Ugrian populations? Today it is difficult to get a plain answer to this question because the borders between different ethnic groups have been effaced many population remnants have been transferred etc

There is reason to believe that the broad tapeworm in ancient times had a distribution extending farther west in Europe It is for example known that *D latum* previously occurred in Denmark and even in Ireland The endemics known from the lake districts in French Switzerland and northern Italy are today mainly of historical interest The parasite was introduced by European



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Herman Diedrich Spöring (1701–1747) Professor c Medicine University of Åbo/Turku Finland Portrait b G E Schroder State Medical Board Stockholm Swe den

stated that the mystery of tapeworm anaemia is solved on the whole The tapeworm causes vitamin B_{12} deficiency in the host About half the carriers of *D latum* have a decreased vitamin B_{12} level (with serum values under 100 pg/ml) during the spring season in particular The incidence of manifest anaemia in *D latum* carriers may be estimated at 2% at least

Making a long story short vitamin B_{12} deficiency results because a tapeworm carrier is deprived of large quantities of this substance since the parasite absorbs them from his intestine The chain of evidence is as follows The content of vitamin B_1 in the tapeworm is high on average 2 3 μg/g dry weight (while the corresponding figure for *T saginata* is only 0 046) dried and pulverized *D latum* can be used as a source of extrinsic factor in Castle s test the injection of aqueous extracts of *D latum* induces remission in patients with PA Orally administered radioactive vitamin B_{12} is taken up by the tapeworm The distribution of the radioactive vitamin between the parasite and the host can be estimated with almost mathematical accuracy

The logical consequence of these findings is that under certain circumstances in any event the host does not receive a sufficient amount of vitamin B_{12} Certain observations indicate that scarcity of vitamin B_{12} in the diet enhances the risk of tapeworm PA Furthermore it has been shown that tapeworm carriers showing marked vitamin B_1 deficiency harbour large amounts of tapeworm and/or that their parasite is located high up in the intestine (in the jejunum) although the ileum is the most common site of attachment

It is today generally accepted that the absorption of vitamin B_{12} coupled to the intrinsic factor (IF) occurs by the mediation of a receptor substance which binds IF and which is at least principally present in the ileum Such a receptor substance has recently been isolated from ileal mucosal scrapings from pig at R Grasbeck s laboratory (Marcoullis G & Grasbeck R Biochim Biophys Acta 499 309 1977) This receptor is the object of continued investigations The uptake of vitamin B_{12} in *D latum* occurs by another kind of mechanism without the mediation of a receptor substance

The upper portions of the small intestine do not contain the B_{12} IF receptor In a *D latum* carrier with tapeworm attached to the jejunum the parasite is thus in a position to take up vitamin B_{12} for all it is worth without any competition from the intestine



Ossian Schauman (1862–1922) Professor of Medicine University of Helsingfors/Helsinki Finland National Board of Museums

The proximal portions of the parasite are metabolically the most active and it has been found that their content of vitamin B_{12} is the highest per unit weight of dry substance If the tapeworm is located exclusively distally in the small intestine where receptor substance with its great affinity for IF bound vitamin B_{12} is present the host is in a better position Vitamin B_{12} is absorbed in sufficient amounts and no or only a relative deficiency ensues

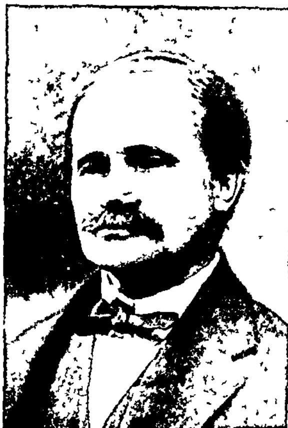
If the gastric production of IF in a *D latum* carrier is reduced it may be assumed that the untoward effect of the parasite on the absorption of vitamin B_{12} is further enhanced That a relative IF deficiency must contribute to the development of tapeworm PA was postulated by us even before vitamin B_{12} had been discovered As previously mentioned patients with tapeworm PA may secrete gastric juice with a normal acidity and in normal amounts and it has moreover been demonstrated

that their gastric juice—unlike that of patients with genuine PA—always contains IF Today quantitative determination of IF is possible and has been performed by J Salokannel (1970) In some cases of tapeworm PA this author observed a marked decrease in IF which was considered to be of essential importance in the pathogenesis of the anaemia In other cases the decrease was more moderate *though probably still a contributory cause of the* disease and in some cases no IF deficiency was noted

The production of IF decreases with advancing age in parallel with an increased prevalence of achlorhydria and gastritis At the same time the risk of vitamin B_{12} deficiency increases in elderly tapeworm carriers Tapeworm PA may however occur in young persons also even in children under 10 years of age in whom the production of gastric juice is abundant and IF secretion is apparently normal too The results of the quantitative IF determinations reported are in agreement with these facts

It was long ago maintained by O Schauman that a genetic predisposition exists for tapeworm PA just as for genuine PA Cases of both forms of PA were reported among close relatives Patients who had suffered when young from tapeworm PA sometimes developed genuine PA at a more advanced age It ...uld be emphasized however that tapeworm PA ...ents who have been cured by expulsion of the ...rm by no means always develop a genuine PA ...ater nor do they necessarily develop PA if they become re infected with *D latum*

The production of IF is subject to genetically determined endogenous influences associated with autoimmune phenomena For such reasons the IF secretion of some individuals ceases completely in the course of time and genuine PA results Others perhaps have a reduced though still sufficient IF production However if such persons are infected with *D latum* they run a great risk of developing tapeworm PA *Expulsion of the worm may cure the* anaemia but after this the IF production in some cases continues to decrease and in the end we may be faced with a patient suffering from genuine PA Summing up any *D latum* carrier may develop tapeworm PA, but presumptive genuine PA patients run a greater risk than others The constitutional genetic disposition postulated by O Schauman thus consists of the same tendency towards a decreased IF production dependent on



Constantin Janicki (1876–1932) Professor of Zoology University of Warsaw Poland

endogenous factors which leads to the development of genuine PA if it becomes total

As already mentioned the most essential cause of vitamin B_{12} deficiency in *D latum* carriers is the great avidity of the worm for vitamin B_{12} The greatest risk of developing vitamin B_{12} deficiency is run by that host whose diet is poor in this vitamin whose IF production is reduced and who harbours the parasite high up in the intestine This constellation of predisposing factors must prevail for a sufficient length of time *That in some cases no* or only a relative vitamin B_{12} deficiency without any manifest PA results is attributable to the fact that the host organism contains a considerable store of vitamin B_{12} which is only gradually depleted It is also likely that periods of better and poorer vitamin B_{12} absorption alternate If the requirement of vitamin B_{12} is increased for some reason or other this may perhaps also contribute to the development of PA

Results have been published which seem to sug

est that the absorption of nutrients other than vi amin B_{12} (folates and other vitamins) may also be mpaired by the presence of *D latum* in the in estine and that changes in the intestinal flora may moreover be involved It may be stated however hat such factors are of minor importance in the pathogenesis of tapeworm PA

Problems connected with the host–parasite rela tionship are of current interest in parasitology The nterference of the broad tapeworm with the B_{12} metabolism of the host illustrates in a unique way the possible development of such an interrelation ship From the standpoint of the broad tapeworm the human intestinal canal is probably the best of all worlds The situation viewed from the standpoint of the host no doubt looks different

For details and references cf B von Bonsdorff *Diphyl lobothriasis in man* Academic Press London 1977

Bertel von Bonsdorff
Helsingfors/Helsinki Finland

BOOK REVIEW

Diphyllobothriasis in man By B von Bonsdorff 189 pages £9.50 Academic Press London New York and San Francisco 1977

It has been said that the Finnish Society of Hematologists should have the broad tapeworm as its mascot In this very recent monograph on Diphyllobothriasis in Man Professor Bertel von Bonsdorff recounts the exciting history of tapeworm anemia Numerous Finnish hematologists in particular the author and his collaborators have solved many such problems and the story of their systematic investigations makes enthralling reading

Few modern hematologists realize that a century has passed since astute clinical observers in Dorpat and Helsinki produced the first proofs that expulsion of the tapeworm from the severely anemic carrier caused remission and healing of the patient s anemia From the days of Runeberg Finnish clinicians have worked systematically and now the problem of tapeworm anemia has been finally solved The author himself has contributed many fundamental observations and performed well planned experiments that are all described in detail besides being beautifully illustrated The book also contains valuable data on the zoology and epidemiology of the broad tapeworm

It is now clear that the parasite if located high in the intestinal tract is able to compete with the host for the uptake of vitamin B_{12} That the worm absorbs the vitamin was proved by the finding that dried and powdered worm was an effective antianemic factor if mixed with intrinsic factor and given to patients with classical pernicious anemia This has now been demonstrated still more elegantly by giving labelled B_{12} to worm carriers and later finding the isotope in the "cranial" part of the worm

The author closes the book with the following remarks "By and large the case of the vitamin robber would thus be solved the way in which the crime has been comitted seems likewise to be clarified " This is clearly a case of true parasitism! The book makes equally interesting reading for zoologists and for highly specialized hematologists or for any doctor interested in general medical problems

Jan G Waldenström

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Salmon Calcitonin in the Acute Treatment of Moderate and Severe Hypercalcemia in Man

Ove Nilsson Sven Almqvist and Bengt E Karlberg

From the Department of Internal Medicine Endocrine Unit University Hospital Linkoping Sweden

ABSTRACT The effect of i v infusions with salmon calcitonin was evaluated in the treatment of acute hypercalcemia in 12 patients Clinical improvement and a less critical level of serum calcium were achieved within 24 hours for eight of the patients, for another two after treatment for 48 hours In malignant conditions (six patients) calcitonin was less effective which could be evaluated within 24 hours In addition to rehydration the rapid onset of action and the lack of side effects make calcitonin a drug of first choice in the treatment of acute hypercalcemia

Hypercalcemic crisis is a serious and acute medical emergency of various etiology (16) In front of the sick patient one can rarely be instantaneously sure of the underlying disorder Therefore it is necessary to start treatment of hypercalcemia immediately and perform the investigations for the underlying disease simultaneously with treatment The most common policy has been to start rehydration in addition to a calcium lowering drug (5 11) Phosphate therapy has been widely used and has proved to be effective in the treatment of hypercalcemia (5 6 14) However i v phosphate treatment may be associated with inherent disadvantages such as a minimum efficient dose and in efficient doses soft tissue calcification and renal failure (2) In recent years several studies have shown a good calcium lowering effect of calcitonin (12 13 17) The mode of action of calcitonin is mainly an inhibition of bone resorption (8) and partly an increase in urinary calcium excretion (3) Synthetic salmon calcitonin in a recommended dose of 1–2 MRC units/kg b wt /hour has been given without serious side-effects and has resulted in a good calcium lowering effect especially if the underlying disorder is primary hyperparathyroidism (12 13) In hypercalcemia caused by malignant disorders the effect has been more ambiguous (12 13 15)

The present study is a review of our experience with salmon calcitonin treatment in acute hypercalcemia of moderate to severe intensity and of various origin We have been particularly interested in the onset of action in order to allow safe and well planned surgery as soon as possible for curable diseases If no calcium lowering effect of calcitonin is seen how soon is one to change the therapy? When inefficient the administration of calcitonin should be rapidly switched to other available forms of therapy

PATIENTS AND METHODS

Synthetic salmon calcitonin (supplied by Sandoz Sweden) was given to 12 patients with symptomatic hypercalcemia (Table I) One patient was treated in another hospital and the others in the University Hospital Linkoping All patients had calcitonin added to infusions with physiological sodium chloride and were thus at the same time rehydrated The doses of calcitonin varied (Table II) The reason for our small doses in some patients was mainly lack of experience with the drug in the beginning Parallel to this treatment patients have undergone diagnostic examinations in order to disclose the primary diagnosis as soon as possible When an underlying malignant disorder was found calcitonin treatment was interrupted and the patients got supplemental therapy with oral phosphate steroids or both Four patients with primary hyperparathyroidism were cured by surgery within 1–3 days after lowering of the serum calcium

Laboratory methods

Serum calcium was analyzed with the standard laboratory technique (flame photometry) using an SMA 12 Auto-Analyzer Our reference values are 2 25–2 75 mmol/l In some patients serum albumin (S Alb) was analyzed with the same technique This made it possible to calculate the so-called corrected S-calcium ($S\text{-}Ca_{corr}$) for these patients using the formula $S\text{-}Ca_{corr}=S\text{-}Ca-0\ 0178\ (S\ Alb-42\ 5)$ (4 10) (Table III)

Statistical methods

Significance was calculated by Student s t test

Table I *Clinical material*
PHPT = primary hyperparathyroidism

Pat. no	Age (y)	Sex	Symptoms	Diagnosis	Initial S-Ca (mmol/l)
1	77	♀	Anorexia, polyuria	Vitamin D intoxication	3 98
2	60	♀	Constipation polyuria	PHPT	3 60
3	77	♀	Constipation polyuria deep depression	PHPT	3 45
4	76	♀	Fever polyuria confusion	Myelomatosis	3 75
5	62	♂	Polyuria confusion	Ca vesicae felleae c metastases	3 92
6	69	♂	Anorexia, polyuria	Myelomatosis	3 72
7	51	♀	Anorexia, vomiting	Ca ovarii	3.50
8	78	♀	Anorexia, confusion	Ca. mammae c metastases	3 90
9	31	♀	Anorexia polyuria vomiting	Vitamin D intoxication	3 85
10	71	♀	Vomiting confusion coma	PHPT	4.51
11	66	♀	Confusion coma	Ca. mammae c metastases	4 75
12	68	♀	Anorexia polyuria	PHPT	3 60

RESULTS

There was a significant decrease in serum calcium after 12 hours of treatment with calcitonin (Table II $p<0.001$) A further decline of serum calcium was observed between 12 and 24 hours ($p<0.05$) A similar decrease was indicated by the albumin corrected serum calcium levels (Table III) Another 24 hours of treatment did not lower serum calcium any further In patient 11 however the treatment had to be continued for 48 hours before she was out of risk owing to a very high initial serum calcium

Table II *Calcium lowering effect of salmon calcitonin*

no	Diagnosis	S-Ca (mmol/l) Initial	After 12 h	24 h	48 h	Calcitonin treatment
1	Vitamin D intoxication	3 98	3 15	3 25	2 82	400 MRC daily for 2 days
2	PHPT	3 60	3 06	3 00	2 95	80 MRC daily for 2 days operation
3	PHPT	3 45	2 92	2 75		400 MRC for 1 day operation
4	Myelomatosis	3 75	3 25			240 MRC for 1 day interrupted after 12 h
5	Ca. vesicae felleae c metastases	3 92	3 42	3 15	3 16	1–2 MRC/kg/h double dose on the second day after indomethacin normocalcemia
6	Myelomatosis	3 72	3 00	2 85	2.90	240 MRC daily for 2 days
7	Ca. ovarii	3 50	3 12	2 84	2 47	240 MRC for 1 day
8	Ca. mammae c metastases	3 90	3 05	2.85	2.50	320 MRC daily for 2 days
9	Vitamin D intoxication	3 85	2 65	2 65	2 55	440 MRC for 1 day then addition of steroids
10	PHPT	4 51	3 28	3 05	3 08	800 MRC 1st day 400 MRC 2nd day operation
11	Ca. mammae c metastases	4 75	4 10	3 45	2 85	350 MRC daily for 2 days
12	PHPT	3 60	2 60			2 MRC/kg/h for 1 day operation
Mean ± S D		3.88±0 39	3 13±0 39	2 98±0 25	2.81±0 25	

Initial vs 12 h: $p<0.001$; 12 h vs 24 h: $p<0.05$; 24 h vs 48 h: n.s

Table III *Calcium lowering effect of salmon calcitonin (S Ca_{corr})*

Pat no	S Ca_{corr} (mmol/l) Initial	After 12 h	24 h	48 h
1	4 01	3 27	–	2 84
2	3 61	3 14	3 05	3 03
4	3 83	3 36	–	–
6	3 64	2 94	2 85	2 93
7	3 50	3 25	3 02	2 59
8	3 98	3 22	2 97	2 67
9	3 89	2 75	2 77	2 63
Mean ±S D	3 78±0 20	3 13±0 21	2 93±0 12	2 78±0 18

Initial vs 12 h: $p<0\ 001$; 12 h vs 24 h: n s; 24 h vs 48 h: n s

Patient 4 was infused with calcitonin for only six hours which decreased serum calcium up to 12 hours The effect was then not considered sufficient and calcitonin treatment was discontinued This patient with myelomatosis was later treated with steroids and phosphate Patient 2 diagnosed as having primary hyperparathyroidism had been treated earlier with three daily infusions of 50 mmol phosphate without any decrease in serum calcium Patient 5 who showed a good response during the first day of treatment did not get any further decrease in serum calcium with a double dose of calcitonin between 24 and 48 hours This patient had a gallbladder carcinoma and was normocalcemic after treatment with indomethacin The clinical condition improved in all patients Four of the patients with diagnosed primary hyperparathyroidism could undergo surgery within five days after the start of calcitonin treatment

Side-effects were not seen and in no case was hypocalcemia induced

DISCUSSION

In the present study we wanted to evaluate the hypocalcemic effect of i v salmon calcitonin on symptomatic hypercalcemia of various origin Although the dose of calcitonin given was low for some patients it was effective in most cases Within 24 hours eight of 12 patients achieved a much less critical level of serum calcium i e about 3 0 mmol/l Serum calcium became almost normal after 24 hours in another two patients

As in earlier reports (12 13) patients with primary hyperparathyroidism tended to respond best to calcitonin treatment probably because of the rapid action on bone resorption (8) In one of the patients with hyperparathyroidism (no 2) it was interesting to observe the lack of a calcium lowering effect of 50 mmol phosphate treatment for 3 days Perhaps a double dose of phosphate would have decreased serum calcium in this patient but a daily dose of 50 mmol is mostly considered effective and less risky (6 7)

In accordance with earlier studies patients with underlying malignant diseases seem to respond less well to calcitonin treatment (12 13 15) possibly because of other mechanisms increasing calcium Prostaglandin like substances have been found in some tumors causing hypercalcemia and there are reports that the prostaglandin inhibitor indomethacin has been effective in normalizing serum calcium (1 9) Our patient 5 with a gallbladder carcinoma remained hypercalcemic during treatment with calcitonin phosphate and steroids but became normocalcemic after oral indomethacin

As stated earlier the origin of hypercalcemia is obscure initially in most cases Yet all patients have to be treated as if they had an underlying curable disease and calcium lowering therapy must be started immediately while a definite diagnosis is sought It therefore seems reasonable to start with calcitonin infusions and as we have shown in this study it is possible to estimate the therapeutic effect already after 12–24 hours of treatment If severe hypercalcemia still persists after 24 hours of calcitonin treatment another form of therapy should be considered especially if clinical and biochemical investigations point to malignancy

Patients with symptomatic hypercalcemia are always dehydrated The initial measure of serum calcium may therefore be higher than the accurate level because of an elevated serum albumin During treatment patients always get large volumes of fluids which lower serum albumin For some of our patients we therefore have calculated the corrected serum calcium We noticed a decrease indicating a more true calcium lowering effect of calcitonin

In summary calcitonin added to rehydration seems to be the therapy of first choice in acute hypercalcemia of different and unknown origin The effect can be evaluated after one day

ment and therapy can later be changed quickly if calcitonin proves ineffective or undesirable

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Ectopic Secretion of Calcitonin

H Mulder and W H L Hackeng

From the Department of Internal Medicine and the Laboratory for Endocrinological Chemistry Bergweg Hospital Rotterdam The Netherlands

ABSTRACT In 7 out of 25 unselected patients (28%) with a malignant disease, the circulating calcitonin level had increased Pentagastrin i v gave no elevation of the calcitonin level comparable to the response in patients with medullary carcinoma of thyroid

Key words Calcitonin ectopic calcitonin pentagastrin
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Ectopic secretion of calcitonin can be defined as the secretion of calcitonin by malignant tumours with the exception of medullary carcinoma of thyroid Ectopic secretion of calcitonin is one of the many types of ectopic protein secretion Beside hormones fragments of hormones or prohormones tumours can also produce other proteins such as carcinoembryogenic antigen α_1 foetoprotein and osteoclast activating factor which is produced by malignant degenerated B lymphocytes such as plasma cells (14 15)

As early as in 1934 Leyton (11) proprosed the secretion of hormones by malignant tumours Lately ectopic secretion of many different hormones i e adrenocorticotropic hormone melanocyte stimulating hormone thyrotropin luteinizing hormone follicle stimulating hormone antidiuretic hormone prolactine growth hormone chorionic gonadotropin somatomammotropin parathyroid hormone calcitonin gastrin gut hormones (vaso-inhibitory peptide and enteroglucagon) and erythropoietin has been demonstrated Rees (16) presented an extensive review on this subject in 1975 In recent reports the ectopic production of the hypothalamic cortico releasing factor (3 13) and α subunits of the glycoprotein hormones (22) is discussed It appears that enzymes too like histaminase (1 2) can be synthesized and secreted ectopicly

The aim of our study was to evaluate the frequency of ectopic calcitonin secretion in carcinoma patients In addition the behaviour of the calcitonin concentration after pentagastrin stimulation was investigated

PATIENTS

The basal serum calcitonin level was measured in 25 patients with a histologically proven malignant disease All patients underwent a pentagastrin stimulation test In 5 patients the serum calcitonin level was measured a second time in the course of the disease The serum calcium and serum phosphate concentrations were measured in 23 patients at the time of the serum calcitonin determination

METHODS

The serum calcitonin concentration was measured with a radioimmunoassay (6) (performed by Dr W Schopman and Dr W H L Hackeng the Endocrinological Laboratory Gemeente Ziekenhuizen Rotterdam) The normal value lies below 400 pg/ml Serum calcitonin was determined before and 1 3 and 5 min after an i v injection of pentagastrin (Peptavlon 0 5 mg/kg b wt) Serum calcium and phosphate concentrations were determined according to Kessler and Wolfman (10) the normal range for these concentrations being 2 2–2 8 and 0 9–1 5 mmol/l respectively

RESULTS

Table I shows that an elevated basal serum calcitonin level was found in 7 patients (28%) It is also clear from the table that pentagastrin stimulation was not followed by a significant change in the calcitonin level in any of these patients ($\Delta CT/CT \times 100 = +20$) The same result was obtained in the patients with normal initial levels ($\Delta CT/CT \times 100 = +26$)

Serum calcium and phosphate were not correlated to the serum calcitonin level ($r = -0\ 137$) (Fig 1)

Table II shows the changes in basal serum calcitonin when two subsequent determinations were made during the follow up of five patients The relation to the individual therapeutic measures is apparent

DISCUSSION

Elevated levels of calcitonin in tissue of tumours derived from neuroectoderm were found in 197

Table I *Serum calcitonin concentration basal and after pentagastrin stimulation serum calcium and phosphate concentrations in patients with a proven malignant disease*

Pat no	Sex	Age (y)	Diagnosis	Calcitonin Basal	1	3	5	$\frac{\Delta CT}{CT} \times 100$	S-Ca	$S\ PO_4$
1	♀	69	Oat-cell carcinoma of the lung	700	790	750	700	+13	2 13	1 32
2	♂	59	Adenocarcinoma of the colon	630	610	710	640	+16	2 18	1 26
3	♂	78	Undifferentiated carcinoma of the lung	630	630	760	890	+41	2 90	1 10
4	♂	70	Adenocarcinoma of the lung*	560	650	650	680	+22	–	–
5	♀	62	Grawitz tumour	550	–	400	–	–	2 33	1 12
6	♀	55	Oat-cell carcinoma of the lung	470	520	410	420	+11	1 98	1 16
7	♀	49	Adenocarcinoma of the stomach	460	480	490	530	+15	2 33	1 08
8	♂	63	Adenocarcinoma of the lung	260	290	310	290	+19	2 17	0 68
9	♀	50	Adenocarcinoma of the mamma	240	140	140	70	−44	–	–
10	♀	76	Adenocarcinoma of the mamma	230	270	250	–	+17	2 50	1 22
11	♀	63	Oat-cell carcinoma of the lung	230	240	120	200	+4	2 05	1 08
12	♂	66	Squamous carcinoma of the lung	230	190	250	220	+9	2 39	1 37
13	♂	57	Squamous carcinoma of the lung	210	200	150	180	−4	2 22	1 07
14	♂	56	Oat-cell carcinoma of the lung	190	220	220	200	+16	1 78	1 14
15	♂	53	Squamous carcinoma of the oesophagus	190	230	100	–	+21	2 50	1 24
16	♂	81	Adenocarcinoma of the lung	190	250	170	200	+32	2 52	1 14
17	♀	63	Lymfosarcoma	170	140	90	–	−18	2 37	1 19
18	♂	50	Carcinoid of the lung	150	110	120	210	+40	2 35	1 46
19	♂	63	Adenocarcinoma of the oesophagus	130	140	190	160	+46	2 43	1 11
20	♂	64	Squamous carcinoma of the lung	130	120	70	110	−8	2 43	1 22
21	♂	70	Oat cell carcinoma of the lung	130	30	150	90	+15	2 51	1 08
22	♂	62	Plasma cell leukemia	120	170	150	100	+42	3 10	1 34
23	♂	83	Oat-cell carcinoma of the lung	100	80	30	30	−20	2 15	1 27
24	♂	62	Oat cell carcinoma of the lung	90	120	90	110	+33	2 43	1 17
25	♂	76	Adenocarcinoma of the pancreas	50	90	130	–	+260	2 43	1 17

* Metastases to the skeleton

9) and 1973 (21) Milhaud et al (12) found in 1974 increased serum calcitonin levels in 18 of 35 patients (51 %) with tumours of cells assumingly originating from the neural crest (non medullary thyroid tumours) Percentages of ectopic calcitonin secretion comparable to our results are reported in the literature (Table III) Silva et al (18) found hypercalcitoninaemia of thyroid origin in a patient with adenocarcinoma of the lung Radiotherapy resulted in a decrease in the calcitonin level Some authors suggest that hypercalcitoninaemia is often associated with the presence of bone metastases (4 17) but this is not borne out by our results Hypercalcitoninaemia basal or after stimulation is a sine qua non for diagnosing medullary carcinoma of thyroid But as shown in the recent few years hypercalcitoninaemia is not pathognomonic for medullary carcinoma Very high levels of calcitonin are seen only in medullary carcinoma In the case of slightly or moderately elevated levels one can probably distinguish between C-cell and ectopic calcitonin secretion by using a stimulation test Hennessy et al (7) found that the pentagastrin stimulation test gave the best calcitonin response in patients with medullary carcinoma of thyroid We found in six subjects with medullary carcinoma that the average rise of serum calcitonin compared with basal level was 1 685 % (13) Using the same radioimmunoassay in seven patients with a different type of carcinoma accompanied by elevated calcitonin levels pentagastrin stimulation resulted in a mean rise of only 20 % in this series

To establish ectopic hormone secretion one

Table II *Changes in serum calcitonin concentration in the course of the disease*
I = first II = second determination

Pat no	Therapy	Basal calcitonin I	II	Interval between I and II
1	Surgery	700	380	3 weeks
6	Radiotherapy	470	380	2 months
8	None	260	380	3 months
10	Surgery	230	170	3 weeks
19	Radiotherapy	130	90	2 months

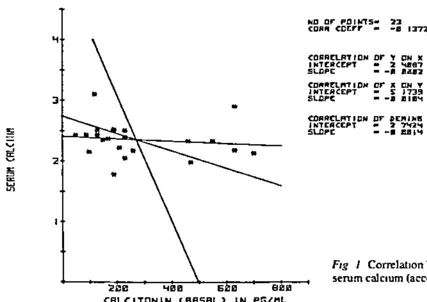


Fig 1 Correlation between serum calcitonin and serum calcium (according to L Deming)

must meet some of the following criteria (16) Association of the presence of a tumour with elevated hormone levels fall in hormone levels after removal of the tumour maintenance of elevated hormone level following extirpation of the normal gland of origin demonstration of an arteriovenous gradient of hormone levels across the tumour demonstration of hormone in tumour tissue and demonstration of secretion in vitro All four persons whom we reinvestigated after tumour mass lowering therapy showed a declining calcitonin level The

Table III *Ectopic calcitonin (CT) secretion reported in the literature*

Ref no	No of pats	Kind of malignacy	Ectopic CT (%)
5	28	Metastatic breast carcinoma	82
5	13	Localized breast carcinoma	8
5	17	Post curative mastectomy	18
4	46	Unselected	46
17	Unknown	Bronchogenic cancer	>50
20	75	Oat-cell and squamous-cell carcinoma of the lung	17
Present series	25	Unselected	28

fifth patient who received no therapy demonstrated an increase although not to a pathologic level The ultimate proof that our seven patients with an elevated immunoreactive serum calcitonin level have an ectopic hormone production has to be delivered The protein detected with the present radioimmunoassay could be one of the calcitonins Sizemore and Heath (19) found in gel filtration studies five immunoreactive calcitonin components in the plasma of medullary carcinoma patients The detected protein could also be procalcitonin one (or several) calcitonin fragment(s) or an unrelated protein

The calcitonin activity cannot be extracted from serum with florisil except in case 3 (in preparation) Chromatography of the sera over a Sephadex G 75 column shows a non-extractable calcitonin activity in the Blue Dextran region (molecular weight ≥60 000) However measuring serum immunoreactive calcitonin level could prove to be a useful method for tumour marking

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Small Cell Carcinoma of the Lung Serum Calcitonin and Serum Histaminase (Diamine Oxidase) at Basal Levels and Stimulated by Pentagastrin

Mogens Hansen Heine H Hansen and Nils Tryding

From the Department of Chemotherapy R II–R V Finsen Institute Department of Internal Medicine C Bispebjerg Hospital Copenhagen Denmark and the Department of Clinical Chemistry Central Hospital Kristianstad Sweden

ABSTRACT Investigations were performed to evaluate the incidence of increased serum calcitonin and histaminase in 79 patients with untreated small cell carcinoma of the lung (SSC) In addition, serum calcitonin was measured following pentagastrin stimulation in 19 of these patients Serum calcitonin was elevated in 54 of 79 patients (68%) 20 patients (25%) having a level usually associated with the diagnosis of medullary carcinoma of the thyroid (MCT) The levels of histaminase, on the other hand, did not differ from the distribution in normals In three of 19 patients undergoing the pentagastrin stimulation test, calcitonin was significantly increased Thus serum calcitonin is frequently elevated in patients with SCC and a positive pentagastrin test is not pathognomonic of MCT

Serological methods for diagnosis and for monitoring therapy in medullary carcinoma of the thyroid (MCT) have been established within the last decade Such methods are particularly valuable in that MCT may be hereditary and occur as part of multiple endocrine neoplasia types 2 and 3 (13 20) *Serum calcitonin is elevated in all patients with* MCT if the tumor has developed to some size (21 22) Even cases with minute tumors can be diagnosed from an increase in serum calcitonin following stimulation e g with calcium (11 21 22) ethanol (7) cholecystokinin (22) or pentagastrin (11 22) The pentagastrin stimulation test is considered the most easy and sensitive of these methods (11 22) Following a calcium infusion serum calcitonin may increase in neoplasms other than MCT (8) while there do not seem to be any reports of falsely positive results with the pentagastrin test

Similarly histaminase (diamine oxidase) is produced by MCT but unlike calcitonin it is particularly increased in patients with metastases (5)

Recently however it has become evident that increased serum calcitonin and serum histaminase in malignancy do not necessarily imply the presence of MCT Both calcitonin (8 15 17) and histaminase (4) may occur in small cell carcinoma of the lung (SCC) which may give rise to a falsely positive suggestion of MCT Accordingly it might be of value to obtain information on the incidence of elevated values of serum calcitonin and serum histaminase in a substantial number of patients with SCC at the time of diagnosis prior to therapy In addition we wanted to know if SCC might give rise to falsely positive results with the pentagastrin test

PATIENTS AND METHODS

All patients in this study had untreated histologically verified SCC according to the WHO classification (14)

Serum calcitonin (2) was determined in 79 patients (analyses performed at the Stockholm Immun Laboratorium) In 70 cases the histaminase (diamine oxidase) (amine oxygen oxidoreductase (deaminating) (pyridoxal-containing) EC 1 4 3 6) was analysed with ^{14}C putrescine as the substrate (23) Baylin (3) has recently shown that the inhibition with antibody to histaminase was identical with histamine or putrescine as substrate

A pentagastrin test (11 22) was performed in 19 patients with measurement of calcitonin in all and histaminase in five Blood samples were drawn in the morning with the patient in the fasting state and samples were drawn simultaneously for analyses of serum creatinine calcium phosphorus and gastrin (19) and plasma glucagon (12) I v bolus injection of 0 5 μg pentagastrin (Peptavlon®/kg b wt was used as stimulation and blood samples were drawn immediately before and 2½ min after the injection The samples were centrifuged at 4 C within a few minutes and serum was kept frozen below −20°C until time of analysis

Dr J Holst performed the analysis of plasma glucagon and Drs J Rehfeld and F Stadil the analysis of serum gastrin

Abbreviations SCC=small cell carcinoma of the lung MCT=medullary carcinoma of the thyroid

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Abbreviations SCC=small cell carcinoma of the lung MCT=medullary carcinoma of the thyroid

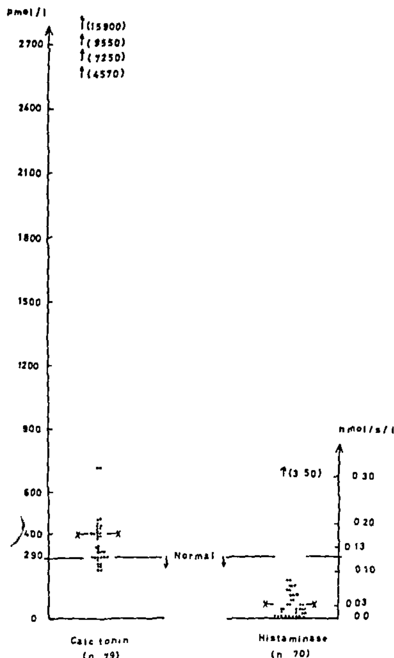


Fig 1 Values of serum calcitonin a histaminase (diamine oxidase) in 79 a 70 patients respectively with SC Upper normal limits and median valu for the patients (×— —×) are indic ed Some patients values (given with parentheses) were markedly higher th those given in the figure

RESULTS

The basal level of serum calcitonin was elevated in 54 of 79 patients (68% 95% confidence limits 57–78%) (Fig 1) The median value was 400 pmol/l It is also noteworthy that in 20 patients (25%) the serum calcitonin value was more than twice the upper limit of normal a level which is in accordance with a diagnosis of MCT (22) None of the patients had impaired renal function judging from normal serum creatinine values Also plasma glucagon and serum gastrin were normal as were serum calciu and phosphorus

The values of histaminase (Fig 1) were elevate in only five patients (7% 95% confidence limi 2–16%) (normal values <0 13 nmol/s/l) moderatel in four and markedly in one The median value wa 0 03 nmol/s/l

In three of the 19 patients undergoing th pentagastrin stimulation test calcitonin was in creased by more than 290 pmol/l at 2½ min after th

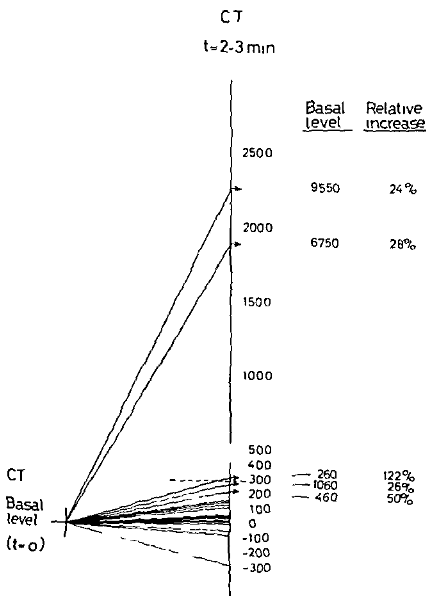


Fig 2 Increase of serum calcitonin (*CT*) within 2–3 min of pentagastrin administration (0.5 µg/kg b.wt.). Ordinate represents the difference (pmol/l) between basal and peak calcitonin levels. The basal level and the relative increase for the five patients with the highest increases are indicated.

injection of pentagastrin (Fig 2) pronouncedly in two (1 800 and 2 300 pmol/l respectively). However, the latter two patients had significantly increased basal levels of calcitonin in serum. Therefore the relative increase in these cases was marginal, while the third patient with an increase of 320 pmol/l had a normal basal level of serum calcitonin giving a relative increase of 122%. In the remaining cases the increase was below 290 pmol/l and the relative increase less than 60%. Thus three of 19 patients (16%, 95% confidence limits 3–40%) had a positive pentagastrin stimulation test. These three patients have died. Autopsy did not reveal any signs of MTC.

In the first five patients submitted to the pentagastrin test, samples were also taken for analysis of serum histaminase following pentagastrin. In none of them did histaminase change significantly. One of these patients showed a significant increase in serum calcitonin.

Three of the five patients with elevated serum histaminase had a concomitantly elevated serum calcitonin level. This frequency does not differ from that in patients with normal values of serum

histaminase, and the only patient with markedly increased serum histaminase had a normal serum calcitonin value

DISCUSSION

Baylin et al (4) found elevated values of histaminase in one third of 25 patients with SCC However as the number of their patients has since increased the proportion of those with elevated values has decreased Thus among 87 patients only 3 had values of serum histaminase definitely above those of 111 controls (9) In only one of 70 patients in our study was serum histaminase definitely above the values of controls Consequently serum histaminase is not significantly elevated in patients with SCC

On the other hand serum calcitonin was increased in 68% of all our SCC patients Milhaud et al (15) found elevated serum calcitonin values in all of thier 5 SCC patients and Coombes et al (8) in 8 of their 11 SCC patients whereas Abeloff et al (1) did not find detectable serum calcitonin in any of their 10 patients An origin of calcitonin from the tumor tissue has not yet been documented However Silva et al (17) have found evidence of ectopic production of calcitonin in SCC and in contrast to lung tumors of other histological types they found pherally elevated values of calcitonin in SCC to of non thyroid origin (18) Finally no physiolog reason for the increase in serum calcitonin was disclosed in this study Thus serum calcitonin is frequently elevated in SCC a phenomenon that appears to be caused by ectopic production

In three of 19 patients undergoing the pentagastrin test the serum calcitonin was significantly increased pronouncedly in two This finding indicates that a positive pentagastrin test is not pathognomonic of MCT Increased calcitonin levels following calcium infusion have previously been demonstrated in one patient with SCC (8)

Light microscopic and in particular electron microscopic studies have suggested that SCC is derived from ectodermal argentaffin cells (6) Endocrine like cells in lung tissue have some properties in common with endocrine APUD cells (10) The malignant counterparts of these cells have been named APUDomas (16) The results presented here support the hypothesis that SCC or at least a group of SCC is related to the APUDomas

In conclusion patients with SCC may give rise to falsely positive results Serum calcitonin at basal levels or stimulated by pentagastrin or calcium infusion is not a specific diagnostic method for MCT This does not however detract from the usefulness of serum calcitonin measurements in the screening of kindreds with multiple endocrine neoplasia

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Bone Mineral Loss during Maintenance Hemodialysis

H Rickers A H Nielsen R Smith Pedersen and P Rødbro

From the Departments of Clinical Physiology and Nephrology
Ålborg Hospital Ålborg Denmark

ABSTRACT With the aim of investigating bone mineral loss during maintenance hemodialysis (MHD), bone mineral content (BMC) was measured by means of two-dimensional scanning photon absorptiometry in 47 chronic renal failure patients on MHD 13 females (age range 22–50 years, mean dialysis duration 189 days) and 34 males (age range 23–69 years, mean dialysis duration 449 days) Measurements were carried out in most patients three times at an interval of 6 months Initial mean BMC values were for both sexes significantly lower than normal but did not correlate to duration of MHD The longitudinal measurements demonstrated a highly significant decrease in BMC with time the mean BMC values after 6 and 12 months, respectively, were for females 95 8 and 93 4% and for males 97 3 and 94 4% of the initial values with no significant differences between sexes The fall in BMC did not correlate to duration of MHD, initial BMC value or age In some of the patients a substantial loss of BMC was observed, and it is suggested that these patients in particular may develop severe bone disease A BMC method with high precision is mandatory for selection of such patients

Chronic renal failure is often associated with a multifactorial bone disease known as uremic osteodystrophy in which secondary hyperparathyroidism or osteomalacic changes may dominate

As the state of terminal uremia can be prolonged by maintenance hemodialysis (MHD) severe bone disease with significant clinical morbidity will constitute an important aspect of uremia which in itself may require treatment The use of 1α hydroxycholecalciferol (1α OHD_3) in preventing bone calcium loss during MHD has been recommended by several authors (3 12 15) but this treatment may harm the patient by causing hypercalcemia (15 19) or a possible deterioration of renal function (18) It is therefore important to know the natural history of bone disease during long term MHD before starting a treatment which may be hazardous to the patient

The magnitude of bone mineral loss during MHD is still discussed recent reports on this issue have been equivocal (9 10 11 13 14) possibly influenced by different regional attitudes to dialysis technique (especially dialysate calcium level) medication with vitamin D and its derivatives and phosphate restriction

The purpose of the present study was to investigate the natural history of bone mineral loss by means of cross sectional as well as longitudinal measurements of bone mineral content (BMC) in patients on MHD

PATIENTS AND METHODS

Forty seven consecutive chronic renal failure patients (13 females and 34 males) on MHD were admitted from the Department of Nephrology for investigations Mean age and duration of MHD are given in Table I Females past 50 years were excluded to avoid the effect of a rapid physiological bone loss No patient had received a renal transplant or treatment with corticosteroids anticonvulsants vitamin D or its derivatives The patients were hemodialysed for 4–6 hours 2–3 times weekly using a Cordis Dow model 2 5 dialyser Blood flow was 200 ml/min dialysate flow 600 ml/min The dialysis fluid was deionized tap water dialysate calcium was 3 0 mEq/l (6 0 mg/100 ml) and dialysate magnesium 1 mEq/l the dialysate contained no fluoride The patients received no dietary calcium or phosphate supply and all had taken 3 g of aluminium aminoacetate daily as a phosphate binder in this way the plasma calcium and phosphate levels were kept as close to normal as possible Most patients were studied three times at an interval of 6 months a few only twice at intervals of 6 or 12 months

BMC was measured on both arms by means of two-dimensional scanning photon absorptiometry on the distal part of the forearm using a Gammatec® GT 30

Abbreviations MHD=maintenance hemodialysis BMC=bone mineral content ΔBMC=fall in BMC 1α-OHD_3=1α hydroxycholecalciferol

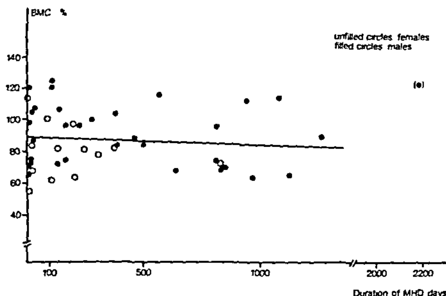


Fig 1 Initial BMC value as a function of MHD duration in 46 patients The slope of the regression line does not differ significantly from zero ($r=0.06$) One patient (●) was excluded from the calculation

apparatus (Firm Gammatec Stormly 16 2890 Hareskov Denmark) with ^{125}I as photon source (8) BMC values were expressed as per cent of sex and age-dependent normal mean and initial BMC values were compared with published reference values (6) Measured precision of repeated measurements in normal subjects over 1 year was with this apparatus 1 4% estimated precision in patients with low BMC 1 7% over 1 year (7)

Student s *t* tests for paired and unpaired data were used for comparison of group means Correlations were evaluated by linear regression analysis

RESULTS

Initial mean BMC values for females and males (Table I) were significantly lower than normal with no significant difference between sexes Fig 1 shows the relationship between initial BMC values and duration of MHD There was no significant correlation

The individual results of the longitudinal study of the same patients are given in Fig 2 for females and males for intervals of 6 and 12 months between BMC measurements These results are summarized in Table II All patients showed a highly significant fall in BMC (ΔBMC) with time similar in males and females amounting to 6%/year The data were further analysed by testing ΔBMC as a function of MHD duration (Fig 3) initial BMC values and age This analysis revealed no significant correlation between ΔBMC and these parameters

DISCUSSION

The present study comprises a consecutive series of patients with chronic renal failure caused by various renal disorders—chronic pyelonephritis chronic glomerulonephritis hypertensive nephropathia and polycystic kidneys constituting the majority of the cases Nearly all patients had a glomerular filtration rate below 5 ml/min

The dialysate calcium and magnesium levels in this study correspond on the whole to those at other centres No dietary calcium supply was given

Table I *Initial BMC values (mean ±S D) in 47 patients on MHD*

	N	Age (y) Mean	Age (y) Range	Duration of MHD (d) Mean	Duration of MHD (d) Range	Initial BMC (% of age and sex-dependent normal)	BMC reference value (N=127)	p
Females	13	42	22–50	189	6–826	81±16[a]	100±16 5	<0 001
Males	34	51	23–69	449	10–2176	92±19	100±16 5	<0 02
Total	47	49	22–69			89±19	100±16 5	<0 001

[a] No significant difference between males and females

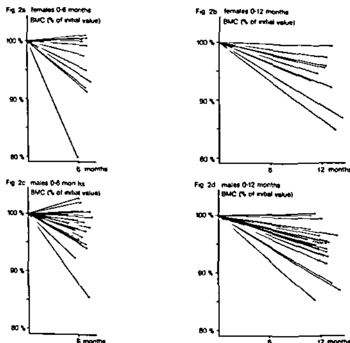


Fig 2 Individual BMC values (% of initial BMC value) for females and males for intervals between BMC measurements of 6 and 12 months

and all patients were treated with a phosphate binder The technique and frequency of dialysis in this department do not differ from those used in other centres

The photon absorptiometry of the forearm is an easy atraumatic and very reliable measurement of BMC (2 8) which reflects total body calcium (5 9) The excellent long term precision—even at low BMC values (<2%) (7)—makes this method particularly qualified for following total body calcium in an individual patient or in a group of patients during treatment whereas the considerable biological variation in BMC (coefficient of variation 16%) makes it less suitable for estimating total body calcium in a single individual In our department we normalize BMC only for sex and age since attempts to normalize it for height weight and bone width did not reduce the biological variation (6)

The initial mean BMC values in our patients were in accordance with the values reported by others (11 13 14 16) significantly decreased in both males and females The relationship however

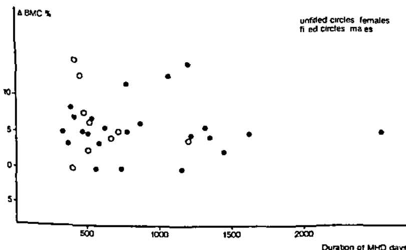


Fig 3 ΔBMC during 12 months as a function of MHD duration in 32 patients

Table II *BMC values (mean±S E M) after 6 and 12 months (% of initial BMC value)*

	N	After 6 months N	%	p	After 12 months N	%	p
Females	13	12	95 8±1 8	<0 05	9	93 4±1 6	<0 005
Males	34	27	97 2±0 7	<0 001	23	94 4±0 8	<0 001
Total	47	39	96 8±0 7	<0 001	32	94 1±0 7	<0 001

No significant difference after 6 and 12 months between sexes
p=Difference from initial significance

between initial individual BMC values and duration of MHD did not show any fall in BMC with increasing duration of MHD suggesting that there is no increase in bone mineral loss during MHD This rather contrasts with the highly significant mean ΔBMC values in both males and females in the longitudinal measurements showing a loss of approximately 6%/year The reason for this discrepancy we think is the considerable intraindividual variation in BMC which might conceal a true fall in initial BMC with duration of MHD This also clearly demonstrates the unreliability of cross sectional studies in estimating changes with time of a parameter with a considerable biological variation (such as BMC) used in relatively small patient groups Such time dependent changes should there f c always be investigated by means of a longi l study Other longitudinal studies of patients MHD did not show a significant fall in mean with time (13 14) but children were included in these studies Furthermore some patients had been treated with 1α OHD_3 or other vitamin D analogues and also the dialysate calcium differed slightly from ours Finally the BMC in those studies was measured with another technique (Norland Bone Mineral Analyzer) which probably has a long term reproducibility considerably inferior to ours (1 4 17) It is therefore possible that our results are partly attributable to the excellent long term reproducibility of our method utilized in longitudinal observations Our results fairly correspond to the findings of other authors (10) who have studied BMC in patients on MHD by means of X ray spectrophotometry demonstrating a mean loss in BMC of 2 4% during 10 months

The present study also showed that some patients were losing bone mineral much faster than others suggesting that as far as calcium loss is concerned our series may contain subgroups of patients with a different natural history It is possible that particularly these rapid losers of bone calcium may develop severe bone disease during MHD and therefore should be treated—e g with vitamin D or its analogues—when separated from the entire group A BMC method with a high precision is mandatory for selection of such patients

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The Relation between Extra- and Intracellular Electrolytes in Patients with Hypokalemia and/or Diuretic Treatment

Thomas Dyckner and P O Wester

From the Department of Internal Medicine Serafimerlasarettet Stockholm and the Department of Internal Medicine University of Umea Umea Sweden

ABSTRACT The relation between extra and intracellular electrolytes has been studied by means of percutaneous muscle biopsies in 107 patients with hypokalemia and/or treatment with diuretics No relation was found between the extra and intracellular concentrations of Na or Mg The serum and muscle contents of K correlated weakly The correlation coefficient tended to be stronger when S-creatinine was normal, total carbonate was between 25 and 30 mmol/l, muscle Mg content was ≥3 95 mmol/100 g fat free dry solids, and when no treatment was given with digitalis and/or diuretics

Key words Potassium magnesium intracellular extra cellular diuretics digitalis

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Since the early 50s much work has been done on the reliability of serum potassium determinations in assessing the intracellular potassium content Less effort has been devoted to the relation between extra and intracellular magnesium The outcome of these investigations as far as potassium is concerned has varied some authors claiming a good correlation between the serum concentration and the intracellular content (6 29) some being unable to find any correlation at all (8 22 23 24 34 50) As for magnesium most authors seem to agree on the discrepancy between its serum concentration and intracellular content (18 30 31 51)

Having studied the initial serum potassium level in patients with acute myocardial infarction in relation to early complications and prognosis we demonstrated an increased frequency of ventricular ectopic beats and ventricular tachycardia in patients with hypokalemia (19) This finding stimulated us to investigate the relation between serum and muscle potassium as well as that between potassium and magnesium (the other main intracellular cation) in serum and in muscle

It is well known that most diuretics augment the excretion of potassium in the urine Whether this results in a cellular potassium deficiency is a matter of debate (5 48) Most diuretics also result in a greatly increased urinary loss of magnesium (17 25 47) The losses can be further accentuated by a concomitant secondary hyperaldosteronism (51) which is fairly common in patients with edema and heart failure (57)

Magnesium deficiency has been demonstrated to influence the potassium balance in a great number of animal studies and also in some case reports (18 30 34 37 43 45 51 54) Magnesium acts as a coenzyme to stimulate the action of Na–K–ATPase which is necessary to keep the sodium pump functioning A modest decrease in the activity of Na–K–ATPase will lead to a cellular accumulation of sodium and a loss of potassium with an unchanged serum potassium level provided the renal function is normal This is what happens in digitalis intoxication digitalis being a well known inhibitor of Na–K–ATPase As magnesium is a necessary stimulator of this enzyme (46) a decrease in magnesium concentration should lead to the same result i e a loss of cellular potassium independent of the serum potassium concentration

The aim of the present study was to investigate the relation between extra and intracellular magnesium and potassium in patients with hypokalemia and/or on diuretic treatment

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Abbreviations CHF=congestive heart failure AHT=arterial hypertension LD=liver disease OD=other diseases FFDS=fat free dry solid(s) K/s=serum potassium K/m=muscle potassium K/ic=intracellular potassium Mg/s=serum magnesium Mg/m=muscle magnesium Mg/ic=intracellular magnesium H_2O/ec=extracellular water H_2O/m=muscle water H_2O/ic=intracellular water Cl/m=muscle chloride Cl/s=serum chloride Na/m=muscle sodium Na/s=serum sodium

Table I *Age (mean ± 1 S D) and sex distribution and some clinical data on the patients*

Diagnosis	No of pats Total	♂	♀	Mean age (y)	On diuretics	On digitalis
CHF	57	15	42	72 8±8 8	54	48
AHT	28	11	17	67 1±12 1	26	1
LD	14	7	7	56 4±9 9	2	1
OD	8	3	5	62 8±17 0		2

PATIENTS

During a period of 2½ years muscle biopsies were taken from 110 patients admitted to the Medical Department Serafimerlasarettet The patients were selected mainly on account of low serum potassium level or diuretic treatment with or without hypokalemia (<3 5 mmol/l) In three of these patients the amount of muscle tissue obtained was not enough for adequate electrolyte determination Of the remaining 107 patients (36 men and 71 women) 62 had hypokalemia on admission In five of these this observation could not be verified later Of the hypokalemic patients 44 were on diuretic treatment Another 38 patients were being treated with diuretics and were normokalemic Seven patients were normokalemic without receiving diuretics the indications for muscle biopsy in this small group were cardiac arrhythmias in 4 patients and muscular weakness in 3

The mean age in the whole patient group was 68 4±11 9 years (men 65 9±11 0 women 69 7±12 2) Table I presents the patients with respect to diagnosis age sex and treatment with digitalis and/or diuretics

About half of the patients (15 men and 42 women) had ongestive heart failure (CHF) All of these 57 patients basal pulmonary rales on admission and pulmonary vascular enlargement on X ray 46 were on digitalis and diuretics 8 on diuretics without digitalis and 2 on digitalis without diretics one patient was untreated Serum creatinine values were below 120 μmol/l in 35 of the patients with CHF 121–200 μmol/l in 16 and above 200 μmol/l in 6

Twenty-eight patients 11 men and 17 women had arterial hypertension (AHT) Two patients were untreated the others were on diuretics one however for a week only one was on digitalis Blood pressure on admission was >160/95 mmHg in 19 of the patients treated for hypertension <140/90 in 4 and 140/90–160/95 in 5 S-creatinine levels of ≤120 μmol/l were found in 24 of the hypertensive patients two had values between 121 and 200 μmol/l and two in excess of 200 μmol/l

Fourteen patients 7 men and 7 women (mean age 53 9±9 6 and 59 0±10 2 years respectively) had liver disease (LD) All except one a female were alcoholics 9 with raised S ASAT and S ALAT on the day of biopsy 2 with clinical signs of liver cirrhosis Three of the alcoho' patients died one of pancreatitis and aspiration one of bleeding from esophageal varices one of digitalis intoxication The patient who was not alcoholic had undergone an intestinal resection on account of obesity and the picture had been complicated by jaundice and muscular weakness

The small group of patients with other diagnoses (OD) consisted of three with cardiac arrhythmias one with angina pectoris one with cerebral embolism one with vasovagal syncope one with diabetes mellitus and one who vomited for unknown reasons

METHODS

Muscle biopsies were performed in the morning after an overnight fast by percutaneous needle biopsy using a technique developed by Bergström (6) All specimens

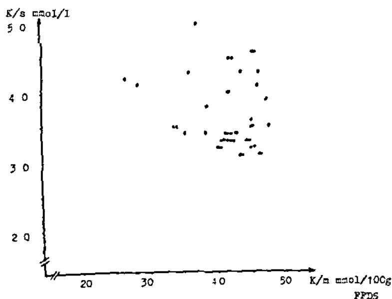


Fig 1 Relation between potassium in serum and in muscle

Table II *Relation between serum and muscle potassium with regard to S creatinine muscle magnesium content total carbonate and presence or absence of treatment with digitalis or diuretics*

	N	Correlation coefficient	p
All pats	107	0 22	0 05
S-creatinine (μmol/l)			
≤120	76	0 41	0 01
>120	31	0 00	n s
Mg/m (mmol/100 g FFDS)			
≥3 95	64	0 39	0 01
≤3 94	43	0 21	n s
Total carbonate (mmol/l)			
25–30	59	0 36	0 01
<25 and >30	38	0 00	n s
Not on digitalis	56	0 38	0 01
On digitalis	51	0 20	n s
Not on diuretics	25	0 49	0 02
On diuretics	82	0 24	n s

were taken from the lateral portion of the quadriceps femoris muscle 15–20 cm proximal to the knee The muscle tissue (20–40 mg) was placed on a piece of quartz glass and carefully dissected free from all visible fat and connective tissue All traces of blood were wiped off by rolling the specimens on the piece of quartz glass The remaining muscle tissue was immediately transferred to a preweighed platinum hook and repeatedly weighed on a Cahn 4700 electromagnetic balance The original weight of the sample was obtained by extrapolation to zero time The muscle tissue was then dried in an oven at 90°C to constant weight and the water content was calculated by subtracting the dry weight from the extrapolated wet weight

Neutral fat was extracted by placing the platinum hook with adhering dry muscle tissue in a quartz tube with 10 ml of redistilled petroleum ether for 2 hours The platinum hook with the muscle tissue was reweighed after drying in the oven for another 2 hours and the fat content was obtained by subtracting the fat free dry solid (FFDS) weight from the weight before extraction

The electrolytes were extracted from the muscle tissue by treatment with 1N nitric acid for 15 hours in quartz tubes A Varian Techtron atomic absorption spectrophotometer was used to determine the contents of sodium potassium and magnesium To minimize the interferences from iron and phosphorus an excess of these ions was added to the diluting solutions for standards and samples as described by Bergstrom et al (7) Coefficients of variation Na 1 0% K 2 5% Mg 2 5%

Chloride was determined indirectly by atomic absorption spectrophotometry An excess of silver nitrate was added to the electrolyte eluate prepared according to Bergstrom et al (7) which was kept in a dark place for 12 hours afterwards After centrifugation the silver content in the supernatant was measured The chloride content was obtained by fitting the obtained silver value into a standard curve where known amounts of chloride had been precipitated by silver nitrate The interference from binding of silver to protein was judged to be insignificant as the protein concentration of the electrolyte eluate was negligible The amounts of silver and halogens in muscle tissue were likewise considered to be of no importance in this respect Coefficient of variation 0 6%

The amount of extra and intracellular water was calculated by the chloride method (24) This method presupposes that chloride is freely diffusible across the cell membrane and distributed according to Nernst s equation (14)

$$E = -\frac{RT}{F} \ln \frac{Clec}{Clic}$$

The resting membrane potential in normal humans has been measured to be 87 2 mV (9) which using Nernst s equation gives a ratio of 26/1 between the extra and the intracellular chloride concentrations

The extracellular chloride concentration (Clec) was estimated from the plasma chloride concentration (Clp) using a correction for the Donnan factor and the plasma water content (H_2O_p) according to Eisenman et al (21)

$$Clec = \frac{Clp \times 1\,000}{0\ 96 \times H_2O_p}$$

Knowing the total water content the total amount of chloride the extracellular chloride concentration and the ratio of the chloride concentrations between the extra and the intracellular compartments it is possible to calculate the water content in the extracellular space The intracellular water content is obtained by subtracting the extracellular water content from the total amount of water

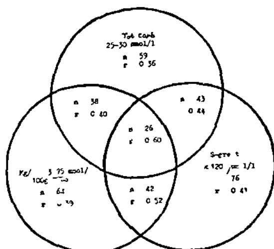


Fig 2 Relation between potassium in serum and in muscle with regard to S-creatinine total carbonate and muscle magnesium content

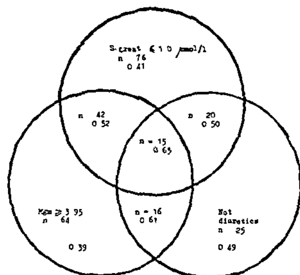


Fig 3 Relation between potassium in serum and in muscle with regard to S creatinine muscle magnesium content and medication with diuretics

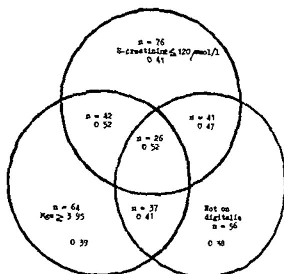


Fig 4 Relation between potassium in serum and in muscle with regard to S creatinine muscle magnesium content and medication with digitalis

$$H_2Oec = \frac{1000 \times Cl_{tot} - H_2O_{tot} \times Clic}{Clec - Clic}$$

$$H_2Oic = H_2O_{tot} - H_2Oec$$

The intracellular electrolyte concentrations are calculated by subtracting the amount in the extracellular space from the total amount of the electrolyte and dividing the result by the amount of water in the intracellular space When estimating the extracellular concentration of the electrolyte it is assumed that it is equal to the plasma concentration

$$Eic = \frac{1000 \times E_{tot} - H_2Oec \times Ep}{H_2Oic}$$

As a basis for reference we used 100 g FFDS

The serum electrolytes were determined by routine laboratory methods Na and K by flame photometry Mg by atomic absorption spectrophotometry Cl by titration with $AgNO_3$ Creatinine and total carbonate were determined by autoanalyzer technique

Statistics

The formula for arithmetic means has been used to calculate the mean values The correlation coefficients were calculated by the formula for linear regression The *t* test was used to determine if there were any significant differences between the mean values in the different groups The Fisher *Z* was used to test the significance of the correlation coefficients

RESULTS

The results are presented in Figs 2–8 and Tables II–VIII The figures and Table II express the relation of electrolytes and water between different compartments Tables III–VIII contain mean values of electrolytes and water in different subgroups of the total patient series The serum concentrations are expressed in mmol/l, the intracellular concentrations in mmol/kg intracellular water the muscle electrolyte contents in mmol/100 g FFDS and the water in g/100 g FFDS

Fig 1 shows the relation between serum potassium (K/s) and muscle potassium (K/m) in the undivided patient group A weak correlation ($r=0\ 22$ $p<0\ 05$) was found The coefficient was somewhat higher for men than for women and was also higher in younger than older age groups (<60 years 0 51 $p<0\ 02$) Dividing the whole group into different diagnostic groups does not give any significant correlation neither does a division into hypo- and normokalemic patients or into hypo- and normomagnesemic

In Table II the patient series has been divided according to S-creatinine level muscle magnesium (Mg/m) total carbonate absence or presence of treatment with digitalis or diuretics The group with a normal S creatinine (≤120 µmol/l) shows a correlation between K/s and K/m ($r=0\ 41$ $p<0\ 01$) in contrast to the group with a high S creatinine which does not show any correlation at all between K/s and K/m ($r=0\ 00$) The group with a Mg/m ≥3 95 mmol/100 g FFDS exhibits a correlation between K/s and K/m ($r=0\ 39$ $p<0\ 01$) again in

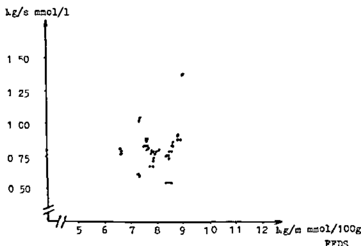


Fig 5 Relation between magnesium in serum and in muscle

contrast to the group with a lower Mg/m (r=0 21 n s)

The group with a normal total carbonate (22–30 mmol/l) does not show any significant correlation between K/s and K/m nor do the patients with a total carbonate outside normal ranges However the group with a total carbonate of 25–30 mmol/l shows a correlation between K/s and K/m (r=0 36 p<0 01) again in contrast to the patients with a total carbonate outside these limits (r=0 01 n s)

Dividing the patient material according to treatment with digitalis there is a correlation between K/s and K/m in the group not on digitalis (r=0 38 p<0 01) The group on digitalis does not show any significant correlation (r=0 20 n s)

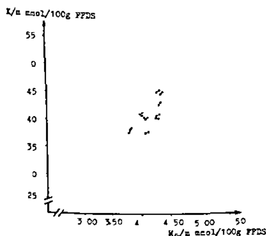


Fig 6 Relation between muscle content of magnesium and potassium

Dividing the series according to treatment with diuretics gives a correlation between K/s and K/m in the group not on diuretics (r=0 49 p<0 01) The group on diuretics does not show any significant correlation (r=0 24 n s)

Figs 2 3 and 4 exhibit the effect on the correlation coefficient between K/s and K/m when the factors in Table II are combined A combination of two factors gives a higher correlation coefficient than one factor alone Combining three positive factors raises the correlation coefficient further (e g r=0 63 p<0 02 for the combination of S-creatinine ≤120 μmol/l Mg/m ≥3 95 mmol/100 g FFDS an no treatment with diuretics) A combination of all four positive factors does not raise the correlation coefficient further (r=0 51)

Fig 5 shows the lack of correlation between serum magnesium (Mg/s) and Mg/m in the whole patient series (r=0 13 n s) This correlation coefficient does not become significant even when groups with a high S creatinine and treatment with digitalis and/or diuretics are excluded nor is there any correlation when the series is divided according to diagnoses normo- and hypomagnesemia normo- and hypokalemia and total carbonate

Fig 6 shows the correlation between K/m and Mg/m in the undivided patient group (r=0 69 p<0 001) This correlation persists even when the division is done according to diagnoses except in the very small OD group

The intracellular potassium concentration (K/ic) correlated strongly to the intracellular magnesium concentration (Mg/ic) (r=0 80 p<0 001) The correlation persisted in the different diagnostic groups

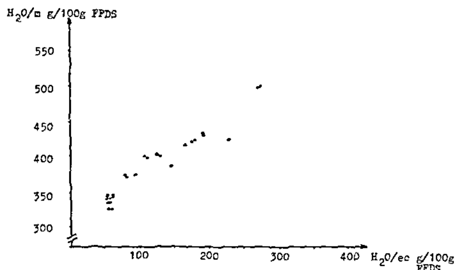


Fig 7 Relation between extracellular water and muscle water content

Fig 7 shows the relation between extracellular water (H O/ec) and muscle water (H_2O/m) There is a highly significant correlation ($r=0.81$ $p<0.001$) in the undivided patient group Subdivision according to diagnoses gives significant correlations in all groups except OD

There was also a good correlation between muscle chloride content (Cl/m) and H_2O/m ($r=0.79$ $p<0.001$) and between muscle sodium content (Na/m) and H O/m ($r=0.82$ $p<0.001$) but no correlation at all between intracellular water content H_2O/ic) and H_2O/m ($r=0.00$)

Fig 8 shows a significant correlation between K/m and H_2O/ic ($r=0.48$ $p<0.001$) Mg/m and H_2O/ic were also correlated to each other ($r=0.49$ $p<0.001$) H_2O/m did not show any correlation to K/m ($r=0.02$ n s) or to Mg/m ($r=0.00$ n s) The serum sodium concentration (Na/s) and Na/m did not show any correlation to each other neither did the serum chloride concentration (Cl/s) to Cl/m

Table III presents the mean values of the electrolytes and water in all patients studied divided into groups according to diagnoses The mean for K/s was higher in the group with CHF than in the group

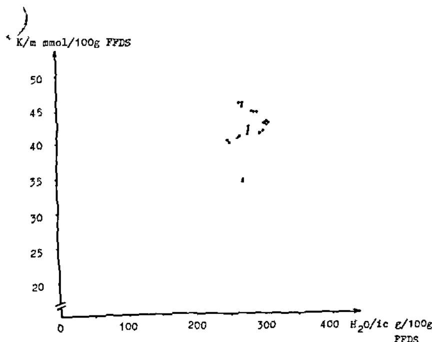


Fig 8 Relation between intracellular water content and muscle potassium content

Table III *Extra and intracellular concentrations and muscle contents of potassium magnesium sodium chloride and water in the four diagnostic groups (mean ± 1 S D)*

Extracellular concentrations given in mmol/l intracellular concentrations in mmol/kg intracellular H_2O muscle content in mmol/100 g FFDS H_2O in g/100 g FFDS

		CHF (n=57)	AHT (n=28)	LD (n=14)	OD (n=8)
K	Serum	3 74±0 58	3 46±0 68	3 54±0 94	3 79±0 49
	Intracellular	146±21 1	150 ±17 3	140±12 3	161±20 3
	Extra + intracellular	41 3±4 86	40 2±4 96	40 2±3 66	44 1±3 24
Mg	Serum	0 83±0 14	0 79±0 13	0 74±0 14	0 87±0 23
	Intracellular	14 4±2 28	15 3±1 90	14 0±2 13	16 2±2 54
	Extra + intracellular	4 02±0 45	4 04±0 39	4 04±0 50	4 36±0 39
Na	Serum	139±5 28	141±3 47	138±8 10	139±4 31
	Extra + intracellular	20 9±8 43	21 0±9 81	22 3±8 91	21 2±7 37
Cl	Serum	98 5±8 76	97 5±7 24	94 9±17 2	95 3±8 15
	Extra + intracellular	15 6±8 83	16 2±10 9	15 7±9 72	14 6±4 99
H_2O	Extracellular	126±73	127±81	126±64	126±43
	Intracellular	284±46	267±36	291±35	275±40
	Extra + intracellular	410±56	394±71	410±56	400±57

with AHT (p<0 05) There was no difference statistically between the mean values for Mg/s in the various groups

The mean values for K/ic and K/m were higher in the group with OD than in the other three diagnostic groups These differences achieved significance be tween K/ic in OD and LD groups and between K/m in OD AHT and LD groups

The mean values for Mg/ic did not differ signifi cantly between any of the diagnostic groups Mg/m was higher in the OD group than in the CHF group (p<0 01) The mean values for H_2O/m Cl/m Na/m and H_2O/ec did not differ significantly be tween the diagnostic groups

When the whole study group is divided into normokalemic and hypokalemic groups irrespective of diagnoses (Table IV) the former group has a higher concentration of Mg/s (p<0 05) and Cl/s

Table IV *Extra and intracellular concentrations and muscle contents of potassium magnesium sodium chloride and water in the total group divided into subgroups according to potassium and magnesium in serum (mean ± 1 S D)*

Amounts in the same units as in Table III

		Normokalemic (n=50)	Hypokalemic (n=57)	Normo-Mg (n=59)	Hypo-Mg (n=48)
K	Serum	4 22±0 41	3 14±0 35	3 75±0 70	3 52±0 59
	Intracellular	151±20 5	145 ±18 4	149±21 4	146±17 0
	Extra + intracellular	42 3±4 95	40 0±4 18	41 3±5 18	40 8±4 00
Mg	Serum	0 84±0 13	0 78±0 15	0 90±0 11	0 70±0 10
	Intracellular	14 8±2 38	14 7±2 11	14 9±2 38	14 5±2 03
	Extra + intracellular	4 11±0 41	4 00±0 45	4 09±0 48	4 00±0 36
Na	Serum	140±3 92	138±6 20	139 3±5 80	139 ±4 66
	Extra + intracellular	21 4±8 51	21 0±8 90	21 0±8 52	21 3±8 97
Cl	Serum	102±6 94	94 0±10 7	97 8±10 3	97 2±9 35
	Extra + intracellular	17 3±9 60	14 2±8 69	15 5±8 95	15 9±9 62
H_2O	Extracellular	132±71	121±73	125±72	1[illegible]7±72
	Intracellular	282±47	276±38	279±44	279±40
	Extra + intracellular	414±57	397±62	404±55	406±67

Table V *Extra- and intracellular concentrations and muscle contents of potassium magnesium sodium chloride and water in the total group divided into subgroups according to muscle content of potassium and magnesium (mean ± 1 S D)*

Amounts in the same units as in Table III

		K/m		Mg/m	
		≥40 0 (n=71)	≤39 9 (n=36)	≥3 95 (n=64)	≤3 94 (n=43)
K	Serum	3 80±0 60	3 34±0 66	3 68±0 64	3 58±0 69
	Intracellular	151 ±16 9	141 ±22 8	151 ±15 9	142 ±23 0
	Extra + intracellular	*43 7±2 50*	*35 9±3 53*	*43 1±3 35*	*38 1±4 77*
Mg	Serum	0 81±0 14	0 80±0 16	0 82±0 16	0 79±0 13
	Intracellular	14 8±2 03	14 6±2 61	15 4±1 78	13 8±2 49
	Extra + intracellular	4 22±0 36	3 71±0 36	4 32±0 29	3 65±0 27
Na	Serum	140±4 10	138±6 99	140 ±3 92	138±6 77
	Extra + intracellular	18 6±7 00	26 2±9 55	19 3±7 10	23 9±10 0
Cl	Serum	99 3±7 28	93 9±12 9	98 4±7 65	96 3±12 4
	Extra + intracellular	12 9±6 90	21 1±10 80	13 6±7 57	18 7±10 6
H_2O	Extracellular	103±55	164±83	109±59	152±82
	Intracellular	291±36	256±44	285±36	270±49
	Extra + intracellular	393±51	428±70	394±52	421±68

($p<0$ 001) than the latter The mean values for Na/s K/m Mg/m Cl/m, K/ic and Mg/ic however do not differ significantly between the groups

When the division is done according to normo- and hypomagnesemia (Table IV) there is no significant difference between the mean values for K/s but a higher mean value in the normomagnesemic group Neither the means for K/m and Mg/m nor for K/ic and Mg/ic differ significantly

In Table V the patient material is divided into one group with a higher K/m and another with a lower K/m The former had significantly higher mean values for K/s ($p<0$ 001) Cl/s ($p<0$ 01) K/ic ($p<0$ 02) and Mg/m ($p<0$ 001) The latter had significantly higher mean values for H_2O/m ($p<0$ 01) and H_2O/ec ($p<0$ 001) but lower H_2O/ic ($p<0$ 001)

Studying the patients divided into one group with a higher Mg/m (≥3 95 mmol/100 g FFDS) and one with a lower Mg/m (≤3 94 mmol/100 g FFDS) (Table V) there is no significant difference between the means for K/s or Mg/s The former group had significantly higher mean values for K/m ($p<$ 0 001) K/ic ($p<0$ 05) and Mg/ic ($p<0$ 01) but lower mean values for H_2O/m ($p<0$ 05) and H_2O/ec ($p<$ 0 001) than the latter

Table VI shows the patient series divided into two groups according to medication with digitalis The mean value for K/s was significantly higher ($p<0$ 02) in the group on digitalis The means for Mg/m and for Mg/ic were both higher although not significantly in the group not on digitalis

Table VII presents the patients divided into groups according to S creatinine one with a normal value one with S creatinine of 121–200 μmol/l and one with a value exceeding 200 μmol/l Although the differences are not significant it can be seen

le VI *Extra and intracellular concentrations d muscle contents of potassium magnesium sodium chloride and water in the total group with regard to medication with digitalis (mean ± 1 S D)*

Amounts in the same units as in Table III

		Digitalis (n=51)	No digitalis (n=56)
K	Serum	3 82±0 60	3 49±0 67
	Intracellular	146 ±21 8	149 ±17 2
	Extra + intracellular	41 4±5 21	40 8±4 16
Mg	Serum	0 80±0 14	0 81±0 16
	Intracellular	14 4±4 93	15 0±1 98
	Extra + intracellular	4 01±0 47	4 09±0 40
Na	Serum	139±4 17	139±6 18
	Extra + intracellular	20 8±9 06	21 5±8 40
Cl	Serum	97 8±8 01	97 3±11 3
	Extra + intracellular	15 2±9 33	15 9±9 19
H_2O	Extracellular	122±74	130±70
	Intracellular	285±48	274±36
	Extra + intracellular	407±67	404±54

Table VII *Extra and intracellular concentrations and muscle contents of potassium magnesium sodium chloride and water in three groups of patients with different S creatinine values (mean ±1 S D)*
Amounts in units as in Table III

		Creatinine (μmol/l)		
		≤120 (n=76)	121–200 (n=21)	>200 (n=10)
K	Serum	3 61±0 63	3 77±0 71	3 66±0 79
	Intracellular	147±20 9	152±17 8	146±7 94
	Extra + intracellular	40 9±4 71	41 3±5 39	42 2±2 54
Mg	Serum	0 78±0 15	0 87±0 14	0 86±0 11
	Intracellular	14 6±2 38	15 6±1 92	14 2±0 98
	Extra + intracellular	4 01±0 40	4 19±0 56	4 07±0 37
Na	Serum	139 ±5 21	138 ±5 53	140 ±5 65
	Extra + intracellular	21 3±9 00	20 9±9 00	20 9±5 76
Cl	Serum	97 7±10 4	97 3±9 70	96 8±5 65
	Extra + intracellular	16 0±9 57	15 5±9 60	13 2±4 86
H_2O	Extracellular	128±72	127±84	108±39
	Intracellular	280±43	272±48	287±22
	Extra + intracellular	408±64	399±57	395±23

that K/m rises with an elevated S-creatinine Mg/s is higher in the groups with a high S-creatinine than in the normal group

When the patients are divided into groups on the basis of whether or not they are treated with diuretics irrespective of diagnoses and types of diuretics there is no significant difference between the mean values for K/m Mg/m Cl/m and Na/m nor between those for K/ic H_2O/m H_2O/ec H_2O/ic Na/s and Cl/s

When the patients not on diuretics are divided into a normokalemic and a hypokalemic subgroup K/m is higher in the former than the latter subgroup ($p<0$ 02) Mg/s differs significantly ($p<0$ 05)—the higher mean value being in the normokalemics—but not Mg/m K/ic or Mg/ic Corresponding calculations for the patients on diuretics divided into a hypokalemic and a normokalemic subgroup do not result in any significant differences

Table VIII presents the patients with a normal S creatinine (≤120 μmol/l) on diuretic treatment divided into three subgroups one on diuretics for less than 3 months one for 3 months–3 years one for more than 3 years and for comparison one group not on diuretics This table shows a successive decrease in K/m and Mg/m with increasing duration of treatment with diuretics This is also true for K/s and Mg/s and to a somewhat lesser extent for K/ic and Mg/ic The difference between the mean values for K/m in the normokalemic non-diuretic group and in the group on diuretics for more than 3 years is significant ($p<0$ 02) and so is the difference between the mean values for K/s ($p<0$ 01) Mg/s ($p<0$ 05) and Mg/m ($p<0$ 05) in the same groups

DISCUSSION

Potassium and magnesium are mainly intracellular cations The extracellular amount constitutes only a

Table VIII *Potassium and magnesium in serum (mmol/l) muscle (mmol/100 g FFDS) and intracellular space (mmol/kg intracellular H_2O) with regard to duration of treatment with diuretics (mean ±1 S D)*

	K/s	K/m	K/ic	Mg/s	Mg/m	Mg/ic
Non-diuretic normokalemic group (N=12)	4 23±0 54	43 5±3 46	151±20 6	0 87±0 19	4 27±0 44	14 8±3 11
Diuretics for <3 mo (N=12)	3 74±0 44	43 6±3 84	153±18 8	0 83±0 19	4 06±0 54	14 5±2 39
Diuretics for 3 mo –3 y (N=20)	3 71±0 63	39 9±5 33	145±21 9	0 82±0 12	3 89±0 42	14 4±2 22
Diuretics for >3 y (N=20)	3 54±0.52	39 6±4 53	143±23 1	0 75±0 14	3 96±0 34	14 3±2 41

few per cent of the total body stores Determinations of the serum values commonly used in clinical routine may not be adequate for assessing the body potassium and magnesium status Various factors such as medication hormones pH kidney function intake of minerals etc may influence extracellular or intracellular values or both

As far as potassium is concerned the literature so far has demonstrated widely diverging opinions on the adequacy of the serum potassium concentration as a reliable guide to the diagnosis of potassium deficiency Radioisotope dilution studies have been performed by several investigators on patients with different diseases and on healthy human beings as well as on animals of different kinds The interpretations of results have been somewhat conflicting Moore et al (40) found no correlation between the serum potassium concentration and total exchangeable potassium These findings were confirmed by Flear et al (23) These two investigations expressed total exchangeable potassium on the basis of unit body weight When Leibman and Edelman (29) referred their results to dry body solids, they found a significant correlation between the serum potassium concentration and total exchangeable potassium

A lowered total exchangeable potassium value may reflect a true intracellular potassium deficit a mere loss of cell mass with no alteration in intra llular potassium concentration or a combination these two possibilities (15 42) This might ex n the inability to correlate these parameters in he former two investigations The conclusion one may draw is that the total exchangeable potassium may not be adequate for assessing an intracellular potassium deficiency as it fluctuates with alterations in muscle mass weight and total body water as well as fat content

In 1962 Bergström (6) demonstrated a significant correlation between the serum potassium concentration and the total muscle potassium content from muscle biopsies expressed per 100 g FFDS in patients with renal diseases and patients with diarrhea However the correlation was significant only when serum potassium concentrations of more than 5 mmol/l were excluded As Bergström pointed out this fact probably reflects the inability of the cell to store potassium in excess of the normal content In 1967 however Graham et al (24) were not able to find any correlation between the serum levels of the electrolytes studied and the muscle values Valentin and Olesen in 1973 (50) found no correlation between total exchangeable potassium or intracellular potassium and the serum potassium level

We found a very weak but significant correlation between K/s and K/m The reason for the weaker correlation than that found by Bergstrom could be that our patients suffered from diseases involving medication with agents influencing the intra or the extracellular concentrations of potassium independently or diseases that per se bring about changes capable of influencing this balance

Although there is a statistically significant correlation between K/s and K/m it is obvious that K/s in the individual case is highly unreliable for predicting K/m In their review of factors that may influence the relation between K/s and K/m Scribner and Burnell (44) mention changes in the extracellular pH and possibly changes in renal function In our study we get a significant correlation between K/s and K/m when S-creatinine is within normal limits but not if it is elevated The *r* values also differ significantly We have not measured pH in our patients However the patients with a total carbonate between 25 and 30 mmol/l had a significant correlation between K/s and K/m in contrast to those with values outside these limits

Several animal experiments have demonstrated that magnesium could be of importance for the potassium status (34, 53, 55) Severe experimental Mg deficiency in rats resulted in intracellular potassium deficiency with a maintained K/s The intracellular potassium deficiency could not be corrected by potassium supplementation alone Addition of magnesium was necessary to achieve a normal intracellular potassium level The magnesium deficiency results in an inhibition of the membrane ATPase (46) and thereby prevents the cell from accumulating the abundant extracellular potassium which instead is excreted through the kidneys

Dividing our study population into a hypo- and normomagnesemic group we could not find any differences between the groups in the K/s–K/m correlation This is not surprising as Mg/s is considered to be a poor guide for the assessment of a deficiency state Turning to the muscle content of magnesium we found a significant correlation between K/s and K/m in the group with a higher Mg/m but not in the group with a lower Mg/m These results imply that magnesium deficiency could alter the intra and extracellular potassium balance

Digitalis is a well known inhibitor of Na-K-ATPase and could theoretically influence the relation between the intra- and extracellular potassium. The patients not on digitalis showed a significant correlation between K s and K m, in contrast to those on digitalis. Medication with digitalis also resulted in a significantly higher K s, which is to be expected on account of the inhibition of the Na-K-ATPase. This inhibition, however, has not resulted in any significant changes in the intracellular compartment, although Mg m is lower, but under borderline significance, in the group on digitalis. K m and K/T did not alter at all in our study.

We also investigated the influence of diuretics. The patients not on diuretics, but not those on diuretics, showed a significant correlation between K s and K m. The reason for this may be found in the magnesium action of most diuretics and the fact that many patients on diuretics also were on digitalis.

The lower value between K s and K m in our total series may thus be explained by several factors, such as kidney function, acid-base status, magnesium deficiency, digitalis medication. Thus K s is an uncertain guide to K m unless several factors are considered. We have studied some factors and there are probably other important circumstances for the intra- and extracellular balance of potassium, e.g. hormones. An uncritical use of K s may thus lead to erroneous assumptions as to the total body content of this ion. These conclusions are further supported by the study of Lazzara et al. ([illegible]) who conclude that plasma levels of neither potassium nor magnesium reveal the pathophysiologic significance of these ions. A more recent study by Müller et al. (38) on 20 patients suffering from liver diseases, congestive heart failure and respiratory insufficiency, comes to the same conclusion. All patients in their material had low intracellular concentrations of potassium and magnesium, while the serum potassium level remained normal.

K/T is not significantly correlated to K s in our patients, the correlation coefficient being just below that for the relation between K s and K m. The lower correlation may be explained by the fact that K/T is a calculated value, which includes possible errors in the determination of water, chloride and the assumption of a normal resting membrane potential.

Mg s and Mg m are totally unrelated to each other in our study, a finding that agrees with earlier investigations (1, 11, 17, 28, [illegible]). Mg s is surprisingly constant. It is regulated mainly by the kidneys, which in the event of a deficiency are capable of reducing the excretion of magnesium to very low values ([illegible], 49). The renal threshold for magnesium is near or at the physiological concentration and a rising serum magnesium level will result in a rapid renal excretion of the excess ([illegible]). Furthermore, Mg s is influenced by the amount of serum proteins. Hormones may also be of great importance, mainly PTH and aldosterone ([illegible]). As to Mg s, many authors have demonstrated a decrease in Mg m in cases of magnesium depletion (10, 27, [illegible], [illegible], 29) though some have not been able to confirm these findings (1, 18). A very strong correlation between K m and Mg m has been reported earlier (7). This may reflect changes in the cell mass, both Mg and K being mainly intracellular ions.

H_2O ex, Cl m and Na m were strongly correlated to H_2O m, indicating that extra water is mainly confined to the extracellular space. This conclusion may be false, however, if both sodium and chloride have entered the cell simultaneously, in which case the chloride method will lead to an overestimation of the extracellular space. This in turn will result in an erroneously small intracellular space and a likewise erroneously high intracellular electrolyte concentration. Correlations similar to those above between H_2O ex, Cl m, Na m and H_2O m have been reported earlier (6, 24, [illegible], [illegible]). The correlation between H_2O ic and K m has also been described earlier ([illegible]). As K m and Na m were not correlated to each other, these findings imply that the cell, on losing potassium, regulates its internal electrolyte concentration firstly and mainly by shifting water out from the cell. This seems to be the physiologically right thing to do as it keeps the internal potassium concentration relatively constant, and hence the resting membrane potential. A mere replacement of K with Na and H would lead to a profound reduction of the resting membrane potential. It is probable, though, that this mechanism is operative when H_2O ic cannot be reduced further.

The patients in our study generally have a high H_2O m, H_2O ex, Cl m and Na m compared with normal subjects presented by others (6, 24, [illegible], 50). However, compared with the patients presented by these authors, there are only minor differences in

H_2O/ec Cl/m and Na/m but H_2O/m remains high The high water content in our series is not surprising though as many of the patients were edematous and nearly all bedridden The mean age of our patients is considerably higher than that of the patients in earlier investigations except the study of Litchfield and Gaddie (32) Their patients also show a higher mean value for Na/m than the patients of the other investigations Serverely ill patients have been demonstrated to have an increase of more than 40% in Na/m compared to normal subjects (16) H O/ec had a mean value of about 25% of the wet weight of the muscle specimens which is just below the values presented by us

A high Cl/m may result from poorly dissected biopsies with a high content of connective tissue that however would mean a low H_2O/m as connective tissue has a lower water content than skeletal muscle (20 35) and this is contrary to our results If our biopsies had had a high content of connective tissue there should also be a negative correlation between Cl/m and H_2O/m which is not the case

In the diagnostic groups there were no significant differences between the mean values for K/s except for the group with CHF which had a higher mean than the group with AHT This difference could be explained by treatment with digitalis which is almost entirely confined to the group with CHF The group with normokalemia had a statistically [illegible] mean value for Mg/s than the group with [illegible] However in the total series no corre[illegible]ion existed between K/s and Mg/s although the correlation coefficients were borderline below significance Thus different factors may influence K/s and Mg/s

Dividing the patient material according to S creatinine Mg/m or medication with diuretics did not result in any improvement of the correlation coefficient The group on digitalis however showed a significant correlation between K/s and Mg/s contrary to the group not on digitalis This may be explained by digitalis promoting the egress of both K and Mg from the cell

The groups with LD and AHT had significantly lower mean values for K/m and the group with CHF just below borderline significance compared to the group with OD Mg/m was significantly lower in the groups with AHT and CHF compared to the group with OD This may be the result of the use of diuretics in the other three diagnostic groups with an accompanying urinary loss of potassium and magnesium K/m and Mg/m in the group with OD were well in accordance with the normal values presented by other authors (6 24 50)

Long term treatment with diuretics has been a subject of discussion regarding cellular potassium and magnesium deficiency during the last decade Some authors have found a decreased total potassium content (5) some have not (48) This conflicting evidence per se implies a complex mechanism which may be difficult to foresee in the individual case No significant differences were found between our groups on and not on diuretics This may be explained by the facts that many patients had been on diuretics for only a short time and that the non diuretic group was chosen mainly on behalf of hypokalemia

It is obvious in our study that medication with diuretics results in a continuous loss of both potassium and magnesium The group with normokalemia and no diuretic treatment shows a considerably higher mean value both for K/m and Mg/m than the group on diuretics for more than 3 years The differences are significant for K/m and Mg/m but not for K/ic and Mg/ic though even in these cases there is a continuous decrease in the mean values with increasing duration of treatment

We believe that treatment with diuretics may result in a loss of total potassium and total magnesium However not all patients develop a deficiency state Other factors such as dietary intake of potassium and magnesium factors influencing the absorption hormonal factors alcoholism and simultaneous presence of other diseases may either work in the same or in the opposite direction as medication with diuretics

It may be pointed out that magnesium deficiency frequently develops slowly and often does not appear in serum determinations It can result in a cellular loss of potassium with a normal serum potassium concentration It has been shown (14 34 53 55) that the potassium content falls during experimental magnesium deficiency in spite of adequate supplies of potassium The cellular potassium deficiency could only be corrected when adequate amounts of magnesium were given

We found a weak correlation between K/s and K/m in the undivided patient series However when the patients with a lower Mg/m are omitted the others show a better correlation between these parameters This finding too indicates that mag-

nesium deficiency is the cause of the low K/m and not the other way round

In view of our findings one may speculate that the magnesium ion either directly or indirectly has a profound influence on the cellular electrolyte and water balance a deficit leading to a decrease in potassium and cellular water content and an increase in sodium and chloride content

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Drug-Induced Neutropenia in the Stockholm Region 1973–75 Frequency and Causes

Per Arneborn and Jan Palmblad

From Roslagstull Hospital and Department of Medicine IV Södersjukhuset Stockholm Sweden

ABSTRACT The records of 133 patients, discharged with a diagnosis of "agranulocytosis" (i e blood neutrophil levels $<1\ 0\times10^9$/l) during the years 1973–75 in the Stockholm county region, were reviewed In 45 cases the neutropenia was probably caused by drugs other than cytostatics, giving an annual incidence of drug induced neutropenia of 0 01% The most common drugs were thenalidine, sulfonamides and thyreostatics Only one of the 45 patients died during the neutropenic phase It is concluded that the pattern of drugs causing neutropenia has changed in Sweden compared with studies from the latter half of the 1960s, and only about 40% of the cases have been reported to the authorities

Blood dyscrasias are a common and often serious adverse reaction to drugs In Sweden they constitute 8% of all cases reported to the Swedish Adverse Drug Reaction Committee (2) and 48% of those with fatal outcome (3) Among these blood dyscrasias agranulocytosis has received especial attention (4 11 cf 7) Since previous Swedish reports on the incidence and etiology of drug induced neutropenia covering the period 1966–70 some of the drugs have been used more cautiously e g chloramphenicol and others have been withdrawn e g noramidopyrin (metamizol) At the same time it has been found that some new drugs are able to cause neutropenia e g thenalidine (1 5 10) Hence repeated follow up studies should provide clinically important information

However the incidence of drug induced blood dyscrasias is difficult to estimate if the only source is the active reporting to health authorities This drawback might be overcome to some extent by reviewing the records of all patients discharged with a diagnosis of neutropenia (4 11)

The aim of the present study was to review the incidence and causes of neutropenia in hospitalized patients during the years 1973–75 in the Stockholm county region with special reference to the drug induced neutropenias their etiology and the clinical picture

METHODS

In the Stockholm county region with about 1 490 000 inhabitants all hospital diagnoses are recorded on a computer The records of patients discharged with a diagnosis of agranulocytosis (1967 ICD classification 288 99) during the years 1973–75 were selected by courtesy of the Stockholm County Council Public Health Board Information System and after permission had been obtained from the head physicians of the various departments the records were reviewed Furthermore the records of all patients discharged with a diagnosis of agranulocytosis from one of the participating hospitals (Roslagstull Hospital) during the same years were retrieved manually for comparison

The records were reviewed for presence of neutropenia (reflected as blood granulocyte counts $<1\ 0\times10^9$/l) duration of hospital stay sex and age of the patients intake of drugs preceding the detection of neutropenia other possible coexisting diseases presence and clinical presentation of infections and the general outcome for the patient

Neutropenia was classified as drug induced if a drug known to cause neutropenia had been taken recovery followed its withdrawal and no other disease associated with neutropenia was present (e g primary haematologic diseases connective tissue diseases and viral infections) Drug induced neutropenias were further divided into three groups depending on whether the patient had taken only one drug (group I) more than one probable drug (group II) or drugs causing a dose-dependent neutropenia (cytostatics) The latter group is not considered in the following discussion

RESULTS

During the three years 1973–75 137 patients had been discharged with the diagnosis of agranulocytosis according to the computerized data base Seven additional patients were found in the records collected manually The records of 9 pa

Table I *Additional data on the 45 cases of drug induced neutropenia (groups I+II)*

	No of pats	Days of hospital stay (mean)	Males		Females		Fatal cases
			n	Mean age (y)*	*n*	Mean age (y)*	
1973	18	21 8	6	63 (36–78)	12	54 (25–79)	1
1974	9	27 8	2	54 (46–61)	7	63 (31–80)	
1975	18	32 6	3	60 (33–78)	15	61 (22–86)	
Total	45	26 7	11	60 (33–78)	34	59 (22–86)	1

* Range in parentheses

tients were lost and two patients did not fulfil the criterion of having less than 1×10^9 neutrophils/l thus giving a total number of 133 patients Out of these 133 cases 45 (22 in group I 23 in group II) were probably caused by drugs according to the above mentioned definition Of the others 12 were caused by cytostatics 38 were due to a primary haematologic disease, 13 to some other disease In five cases it could not be decided from the present information whether the neutropenia was caused by

Table II *Drugs involved in the 45 cases of drug induced neutropenia*

Group I=patients who had taken only one drug group II=patients who had taken two or more drugs (all possible neutropenia-causing drugs being listed) Swedish synonyms at present on the market are given in parentheses

	Group I	Group II
Sulfonamides	7	4
Various	2	4
Salicylazosulfapyridine (Salazopyrin®)	2	
Trimethoprim-sulphamethoxazole (Bactrim® Eusaprim® Trimetoprim-Sulfa®)	3	
thyroid drugs	5	1
(Neo-		
/	2	1
ouracil (Tiotil®)	3	
Antihistaminics	4	6
Cyproheptadine (Periactin®)		1
Thenalidine (Sandosten®)	4	5
Phenothiazines	1	13
Levomepromazine (Nozinan®)	1	6
Chlorpromazine (Hibernal® Klorpromex® Largactil®)		5
Perphenazine (Trilafon®)		2
Diuretics		5
Bendroflumethiazide (Salures®)		1
Furosemide (Lasix® Impugan®)		1
Hydrochlorothiazide (Dichlotride® Esidrex®)		1
Mefruside (Baycaron®)		1
Polythiazide (Renese®)		1
Antibiotics		4
Cephalexin (Ceporexine® Keflex® Palitrex®)		1
Doxycycline (Idocyklin® Vibramycin®)		1
Nitrofurantoin (Furadantin®)		1
Rifampicin (Rifadin® Rimactan®)		1
Antiphlogistics		2
Oxyphenbutazone (Tanderil®)		1
Indomethacin (Indomee®)		1
Miscellaneous	5	11
Metamizol (noramidopyrin)	1	
Amitriptyline (Larozyl® *Saroten® Tryptizol®*)		1
Carbamazepine (Tegretol®)		1
Nitrazepam (Apodorm® Dumolid® Mogadon® Nitrazepam®)		1
Salicylics		2
Antimalarial combination (Triquine)	1	
Iodine-containing X ray contrast media		1
Penicillamine (Cuprimine® Penicillamine®)	2	
Etilefrin (Effortil® Pressoton®)		1
Quinine (Kinin)		2
Tolbutamide (Artosin® Rastinon®)		1
Natrium aurothiomalate (Myocrisin®)	1	
Methyldopa (Aldomet® Dopamet® Hyperpaxa®)		1

Table III *Infections noted in the records*
Some patients had signs of more than one type of infection

Skin infections (Staphylococcus aureus)	4
Septicaemia	
Staphylococcus aureus	3
E coli	2
Tonsillitis pharyngitis (necrotizing tonsillitis 2)	12
Urinary tract infections	13
Pneumonia	2
Fever of unknown origin	8
No signs of infection	8

a drug or disease In 20 cases the cause of the neutropenia remained unknown

Based on the 45 cases of drug induced neutropenia presented here the mean annual incidence of drug induced neutropenia during these three years is estimated to be 0 01‰ Only one of these patients died of complications to the neutropenia These 45 patients comprise about 0 06‰ of all admissions to hospital during the same time in this region (mean 236 000 admissions/year)

Table I gives further characteristics of the drug induced cases Table II lists the various drugs involved and Table III the infections described in the records

DISCUSSION

The pattern of drugs causing neutropenia in this study resembles earlier findings in some respects Thus various sulfonamides and antithyroid drugs were still among the most frequently noted causes of drug induced neutropenia

There are some differences however Metamizol (noramidopyrin) previously the single most often incriminated drug cannot be prescribed in Sweden since 1973 and consequently metamizol induced neutropenia was rare in this material the only patient had bought a combination analgesic preparation in another country Similarly granulocytopenias induced by butazones are not common compared with earlier reports (4 7 12)

On the other hand in earlier Swedish studies thenalidine induced neutropenia was not mentioned at all (4 11) Three cases have been reported in 1958 by Adams and Perry (1) but it was not until the early 70s that the neutropenia inducing capacity of thenalidine was more generally recognized (7 11) and after some additional case reports (5 10) thenalidine-containing drugs were withdrawn from the Swedish market in 1976 The effect of this withdrawal on the incidence of neutropenia cannot be further analyzed in the present study It can only be concluded that during 1973–75 thenalidine was the single most common cause of drug induced neutropenia in Sweden

Among the antibiotics trimethoprim sulphamethoxazole was the most often noted cause in this study As a single responsible drug it shared second place with propylthiouracil after thenalidine

The annual incidence of a drug induced neutropenia was 0 01‰ in this study from the Stockholm region This figure is similar to the findings in earlier Swedish studies from other parts of Sweden during the latter half of the 60s (4 11) Hence although prescription habits and the pharmaceutical arsenal have changed since then the incidence of neutropenia is rather stable This could indicate (inter alia) that the propensity to react with neutropenia on a drug regimen might be one of the most important factors in the pathogenesis of this condition Apart from such a conclusion the present study shows that some comparatively new drugs may carry a potential risk of inducing neutropenia Among these is penicillamine It is probable that this drug will be used more frequently in the near future for the treatment of e g rheumatoid arthritis Since connective tissue diseases per se also might be associated with neutropenia such a combination of drug and disease should be carefully observed for possible adverse haematological reactions

Most patients in the present study showed signs of infection during their hospital stay for drug induced neutropenia Urinary tract infection (UTI) was the most common clinical finding However while UTI may have been a result of a diminished host defence during neutropenia it could just as well have been the reason for the drug therapy that resulted in neutropenia Pharyngotonsillitis was the second most frequent type of infection Necrotizing tonsillitis which is often suggested as a classic sign in neutropenic patients was only mentioned in two cases More serious infections such as septicaemia were not frequent comprising only about 10% of all infections noted To some extent this may explain the low mortality rate In earlier studies the mortality has ranged between 70 and 80% (6 8) more recently it has been reported to be around 30% or even lower (4 9 11) In the present study we found only one fatal case out of

Another possible explanation for the low mortality rate noted here apart from the few serious infec tions might be that cases with extremely low neutrophil counts were less common in the present material than in the above mentioned studies No further analysis of this hypothesis is possible since detailed data are not available for comparison Fi nally we have excluded perhaps to a greater extent than previous authors patients with other diseases that may likewise cause neutropenia and those on a cytostatic drug regimen two groups that probably have a higher mortality rate

Previous studies have repeatedly shown that the incidence of drug induced neutropenia reflected as the frequency of reports to the Swedish Adverse Drug Reaction Committee is much lower than the real incidence Thus Westerholm and Reizenstein (11) found that only 20–40% of all their cases had been reported by the attending clinicians By courtesy of the Committee a similar analysis performed on the present material showed that only 18 of the 45 drug induced neutropenic cases had been reported This relatively low frequency of ac tive reporting (40%) justifies further studies of the present kind in order to obtain a more complete picture of how drugs may adversely affect the blood

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C-Reactive Protein, C3, C4 and Properdin during the Jarisch-Herxheimer Reaction in Early Syphilis

Claes Otto Kindmark Halvor Moller and Kenneth Persson

From the Department of Infectious Diseases University of Uppsala Uppsala and the Departments of Dermatolo… and Clinical Virology University of Lund Lund Sweden

ABSTRACT The occurrence of plasma CRP during the febrile response after the first injection of penicillin was followed in 10 patients with early syphilis An increase in CRP was noted after 12 hours with a maximum after 24 hours The appearance of this acute phase protein could not be correlated to cutaneous reaction, increased body temperature or leukocytosis nor were baseline values of CRP correlated to clinical or serological activity of the infection Patients with high levels of CRP prior to treatment suffered the most intense systemic reactions No activation of the complement system during the Jarisch–Herxheimer reaction was detected from sequential determinations of C3 C4 and properdin

Key words C reactive protein complement syphilis
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The Jarisch–Herxheimer (J–H) febrile and exanthematous reaction is common when therapy is started in patients with syphilis The cause of this reaction is not clear but it is believed that a sudden massive phagocytosis of dying spirochetes results in a liberation of endogenous pyrogen from sequestered leukocytes Recently it was reported (1 2) that signs of complement activation had been found Only the classical pathway seemed to be involved There are many similarities between the J–H reaction and the reaction to endotoxin Thus in a limulus lysate test Gelfand et al (2) were able to observe an endotoxin like activity in sera from patients suffering the J–H reaction Endotoxin shock and disseminated intravascular coagulation as seen in gram negative sepsis have not been reported in the J–H reaction

C reactive protein (CRP) activates the complement system in the presence of certain polycations at the C1 level (13 16) and also has a phagocytosis promoting effect on a series of different bacteria (5) and is known to be an acute phase reactant Therefore a prospective study was performed to find out if CRP could be involved in the J–H reaction At the same time complement activation was studied by measurement of C3 C4 and properdin

PATIENTS AND METHODS

Ten patients hospitalized for treatment of syphilis were studied 7 were males and 3 females aged 19–58 years Eight patients were in the second stage of luetic infection with appropriate cutaneous lesions while one patient (no 10) had acquired his infection within the last two years and lacked clinical signs of the disease These 9 patients were all strongly seropositive with both Wassermann Meinicke Kline FTA absorption and TPI tests One patient (no 5) had a seronegative primary syphilis with spirochetes in the genital ulcer

The treatment was started at 8 a m with an i m injection of 0 6 g (600 000 IU) benzylpenicillin procaine This dose was repeated every 24 hours The rectal temperature was registered before the first injection every hour during the first 12 hours after the injection and every other hour during the following 12 hours The patients were observed for any change in their cutaneous lesions ESR was measured before initiation of treatment WBC and differential count were made and blood samples were drawn and collected as EDTA plasma before the first penicillin injection and after 6 12 24 and 48 hours The plasma specimens were stored at −20°C until assayed Quantitative measurement of CRP C3 C4 and properdin was done by electroimmunoassay as described earlier (6 8 10)

RESULTS

A J–H reaction defined as febrile response and/or intensified cutaneous reaction was observed in 9 of the 10 patients (Table I) Four patients had a brisk temperature rise to ≥39°C and five had milder or no temperature reactions The roseola was seen to intensify in two of those with a high temperature and in four of the five patients with less marked temperature reactions The febrile response reached its

Abbreviations J–H=Jarisch–Herxheimer CRP=C reactive protein ESR=erythrocyte sedimentation rate WBC=white blood cell count

Table I *CRP (mg/l) body temperature and roseola during penicillin treatment of early syphilis*

Pat no	Age (y)	Sex	Hours after penicillin 0	6	12	24	48	J–H reaction Neg	≥38 C	≥39°C	Enhanced roseola
1	31	♂	4	3	4	5	4	×			×
2	26	♀	7	8	9	14	9	×			×
3	19	♂	38	36	34	23	12		×		×
4	48	♂	3	3	5	1	5	×			×
5	30	♂	2	2	3	2	22		×		
6	55	♀	11	10	17	40	18			×	×
7	35	♂	23	27	35	75	86			×	
8	58	♂	65	54	63	74	38			×	×
9	57	♀	42	60	82	108	63			×	
10	48	♂	5	4	4	4	3	×			
Mean (pats 1–9)			22	23	28	41	29				

maximum after about 8 hours (range 6–13) The temperature curve was found to vary inversely with the CRP curve (Fig 1) the CRP maximum following 18 hours after the temperature maximum Patients with a brisk temperature reaction had much higher initial CRP values and reacted more intensely with CRP production than those with mild reactions Both groups showed maximum CRP values at the same time after about 24 hours Those with high initial CRP values also had higher ESR (mean 44 mm) than those with low CRP (mean 8 mm) No correlation was found between the lues serology titers and the intensity of the J–H reaction There was a mean increase in WBC from 5 5 to 6 6×10⁹/l but no relation to the extent of reaction was discov

The determinations of C3 C4 and properdin not show any marked changes (Figs 2–4)

DISCUSSION

Following antibiotic treatment of luetic infections a great many patients initially develop the J–H reaction it is mainly observed in primary seropositive syphilis and in secondary syphilis (9 11 14) Of our 10 patients with early syphilitic infection the first penicillin injection induced a febrile response in 7 and an intensified roseola in 2 The patient without any type of reaction was the only one who had passed into the stage of early latent syphilis

CRP repeatedly documented as appearing in several bacterial diseases has been stated to be present regularly in syphilis too (3 15) Further more a positive correlation between the strength of CRP and that of different luetic serologic reactions has been observed (15) With the quantitative method used in the present work appreciable amounts of CRP could not be demonstrated before treatment in all 8 patients with a secondary syphilis Thus an active spirochetal infection even with strong serologic reactions does not by itself induce the production of CRP

In our patients the J–H reaction was regularly associated with a rising CRP The patients who showed the most intense J–H reaction reacted with

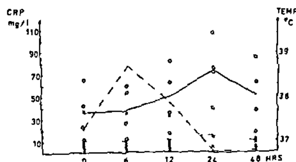


Fig 1 CRP and rectal temperature in the nine patients with J–H reaction Individual and mean CRP values in patients with brisk reaction (O —) and mild reaction (×) =mean rectal temperature in all nine patients

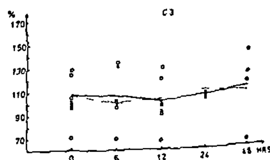


Fig 2 C3 in the nine patients with J H reaction Symbols as in Fig 1

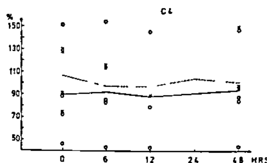


Fig 3 C4 in the nine patients with J–H reaction Symbols as in Fig 1

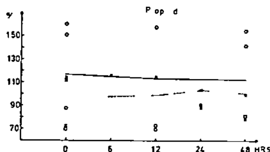


Fig 4 Properdin in the nine patients with J–H reaction Symbols as in Fig 1

the highest CRP production which indicates either that fever induces CRP production or that rise of temperature and increase in CRP are two separate facets of the J–H reaction Studies on experimentally induced fever in man did not disclose a direct linkage between fever and CRP production (4) Accordingly in one of our patients a CRP rise was found although no temperature reaction developed Even the experimental UV light dermatitis in the rabbit although limited is accompanied by an increase in CRP (unpublished results)

As mentioned above an increase in CRP was found in patients with febrile reaction or with only cutaneous reaction Polymorphonuclear leukocytes known to invade the syphilitic skin in the early phase of the J–H reaction (12) could be implicated in both situations giving a fever reaction by liberating endogenous pyrogen and/or possibly intensifying local inflammation by supplying lysosomal enzymes It is possible that some leukocyte factor could induce CRP production On the other hand it has been shown (7) that a patient lacking circulating neutrophils may nevertheless respond to an infection with an increase in CRP Thus the J–H reaction is followed by an increase in CRP but the way in which the CRP production is initiated is still obscure

The fact that those with high initial CRP values suffered the most intense J–H reaction could indicate that CRP was pathogenetically involved CRP is known to enhance phagocytosis and it has recently been found that complexes with CRP and e g protamine and C polysaccharide can activate the complement system in the classical way Changes in the complement factors compatible with activation of the classical pathway have been reported (1 2) In the present investigation there was no definite sign of complement involvement The endotoxin reaction of Gr sepsis regularly causes activation of the alternate pathway An endotoxin like activity has been demonstrated by Gelfand et al (2) but no evidence of alternate pathway involvement was found in their cases Nor was any indication of activation of the alternate pathway observed in the present study Thus despite the presence of endotoxin like activity it has not been possible to show definite signs of activation of the alternate pathway in patients with J–H reaction

Since high initial CRP values were associated with intense J–H reactions it might be possible to predict the intensity of the clinical reaction A positive CRP when the precipitation method of determination is used could be taken as a warning of a brisk J–H reaction

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Effect of Food on the Bioavailability of Bendroflumethiazide

Bjorn Beermann Margaretha Groschinsky Grind and Bjorn Lindstrom

From the Department of Medicine and the Clinical Pharmacology Laboratory Serafimerlasarettet Stockholm and the Division of Drug Trials National Board of Health and Welfare Uppsala Sweden

ABSTRACT Bendroflumethiazide (BFT), 10 mg, was given orally to eight subjects after fasting over night and together with a meal Concentrations of the diuretic in plasma and urine were determined by GLC As judged by AUC_∞ and urinary recovery of BFT, the bioavailability of the diuretic was not influenced by concomitant intake of a meal

Key words Bendroflumethiazide bioavailability diuretics gastrointestinal absorption thiazides
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The effect of a meal on the bioavailability of drugs differs substantially The bioavailability of some drugs is reduced by a meal e g isoniazid (4) while it is unchanged for sulfaisodimidine (6) Other drugs e g hydrochlorothiazide are absorbed to a greater extent when given with food (1) Finally the bioavailability of some drugs e g metoprololol and propranolol (5) is improved despite complete absorption in the fasting state This latter improvement appears to depend on decreased metabolism of the drugs when given with food The uptake of bendroflumethiazide (BFT) is essentially complete when given to fasting subjects (3) It is metabolized to approximately 70% in man The aim of the present study was to examine whether food changes these parameters

EXPERIMENTAL PROCEDURE

Eight subjects aged 18–32 years 7 males and 1 female took part in the study They were healthy according to medical history physical examination and laboratory tests (Hb WBC blood platelets ESR ASAT ALAT S creatinine and S-electrolytes i e Na K Ca^{++} and Mg) Their body weight averaged 71±10 kg ($\bar{x}$±S D)

Each subject participated in two experiments at least 2 weeks apart They were given 10 mg BFT (Salures tablets 5 mg Ferrosan Malmö Sweden) orally after fasting overnight (experiment I) and together with a standardized breakfast (experiment II)

In experiment I the volunteers arrived at the laboratory at 7 30 a m and took the tablets at 8 a m (time zero) They drank 150 ml tap water each hour from −2 to 10 h except at 4 5 and 6 h when they were given a mixture of 75 ml tap water and 75 mg Trilgar (carbohydrates 0 53 g × ml^{-1} Na^+ 30 μmol × ml^{-1} K 0 8 μmol × ml^{-1} Ca^{++} 1 5 μmol × ml^{-1} Mg^{++} 2 5 μmol × ml^{-1} Cl 8 5 μmol × ml^{-1} PO_4^{--} 6 3 μmol × ml^{-1})

On the day of experiment II the subjects likewise arrived at the laboratory at 7 30 a m They were served a standarized breakfast at 8 a m consisting of a cup of 150 ml non sweetened tea 150 ml orange juice 1 boiled egg one roll with butter and four thin slices of cheese When they had finished the juice the egg and one half of the roll they took two BFT tablets and then completed their breakfast At noon they were served ten small Swedish meatballs with sauce mashed potatoes a sallad containing carrots green peas and corn (≈500 kcal) and 150 ml tap water At 2 30 p m they had 150 ml coffee and a Danish pastry At 5 p m chicken meat was served with mashed potatoes mixed sallad described above (≈500 kcal) and 150 ml tap water

In both experiments the subjects were allowed to drink eat and smoke after 10 h if desired

Venous blood samples were collected twice an hour during the first 2 h after drug administration once an hour at 3–12 h and at 24 h

The first urine sample at 8 a m on the day of experiment was discarded Urine was then collected hourly from time zero until 10 h and thereafter at 10–12 h 12–24 h and 24–48 h

METHODS

The plasma and urine samples of each series were analyzed on the same day The concentrations of BFT in plasma and urine were determined by GLC after extractive alkylation according to Beermann et al (2)

The area under the plasma concentration time curve (AUC_∞) was calculated by the trapezoidal rule and extrapolated to infinity The elimination rate constant (K) of the plasma concentration time of BFT was estimated by

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Abbreviations BFT=bendroflumethiazide GLC=gas-liquid chromatography AUC_∞=area under the plasma concentration time curve V_D=volume of distribution f=fraction absorbed

Table I *Plasma concentrations of BFT given without (A) and with food (B)*

Subj no	Plasma peak of BFT: Time (h) A	B	Concentration (ng×ml⁻¹) A	B	AUC$_\infty$ (ng×ml⁻¹ h) A	B
1	2	2	76	103	388	860
2	2	2	56	74	327	435
3	2	1 5	64	61	441	353
4	2 5	2	107	80	552	548
5	2.5	1 5	106	96	601	640
6	2	2	60	67	501	497
7	2	3	72	66	444	423
8	1 5	1 5	77	73	601	640
Mean	2 1	1 9	77	77	470	524
±S D	0 3	0.5	20	15	89	162

No differences are significant

least square regression analysis after logarithmic transformation The apparent volume of distribution (V_D) was determined as

$$\frac{\text{dose } f}{\text{AUC}_\infty\ \lambda}$$

The plasma clearance of BFT was calculated as

$$\frac{\text{dose } f}{\text{AUC}_\infty}$$

The renal clearance of BFT was estimated by dividing the urinary recovery by the AUC$_\infty$ during the corresponding time f was set as 1 0 when BFT was given to fasting subjects (3) f was also set to 1 in experiments where BFT given with food when the urinary recovery of BFT the same as in fasting subjects

Statistical analyses were performed with Student s paired t test

Table II *Urinary recovery of BFT after administration of 10 mg BFT without (A) or with food (B)*

Subj no	Recovery of BFT (mg) A	B
1	2 75	2 80
2	2 10	2 57
3	3 02	3 30
4	2 56	3 08
5	3 73	3 38
6	3 13	3 17
7	3 57	3 39
8	2 75	2 99
Mean	2 95	3 09
±S D	0.53	0 29
	NS	

RESULTS

Plasma levels of BFT

Peak levels of BFT were reached 2 1 h after administration without food and averaged 77 ng×ml⁻¹ The corresponding figures when BFT was given with food were 1 9 h and 77 ng×ml⁻¹ respectively (Table I)

Likewise AUC$_\infty$ did not differ significantly between the two modes of administration (Table I) There was generally a good agreement within the individuals except for subject 1 whose AUC$_\infty$ amounted to 388 and 860 ng×ml⁻¹ h respectively when BFT was given without and with a meal A pharmacokinetic analysis showed that V_D did not differ between the two occasions (1 43 and 1 34 l×kg⁻¹ respectively) The plasma BFT half life was 2 7 h in the first experiment and 5 6 h in the second which could be explained by lower non renal clearance of BFT 100 compared with 330 ml min⁻¹ The renal clearance was essentially the same on the two occasions amounting to 100 and 70 ml min⁻¹ respectively

Urinary excretion of BFT

The urinary recovery of BFT during the first 48 h after administration without or with food averaged 2 95 and 3 09 mg respectively (Table II) The difference is not significant

DISCUSSION

The present results show that the absorption rate of BFT is not changed by concomitant intake of food

The possibility of reduced gastrointestinal uptake of BFT under postprandial conditions can be ruled out taking into account the similar urinary recovery of BFT in the two experiments

In one subject (no 1) AUC$_\infty$ was doubled in the experiment when BFT had been given with food The cause appeared to be a reduced non renal clearance of BFT which was also reflected in a prolonged elimination rate of BFT The lack of such changes in the other subjects suggests that the reduced non renal clearance of subject 1 was secondary to other factors than administration of the drug together with food

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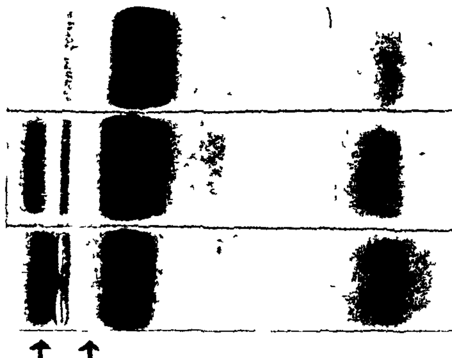


Fig 1 Lipoprotein electrophoresis anode to the right Top normal serum Middle PVP (5 µl/ml serum) added to normal serum showing one abnormal band Bottom serum from patient 1 showing two abnormal bands (arrows)

abnormal band only increased Consequently experiments with serum lipoprotein electrophoresis were made on normal serum (in vitro) the *broad* abnormal band was found after adding Insipidin Retard (concentration 5 µl/ml serum) (Fig 1) while no abnormality was observed when a vasopressin preparation without PVP (Minurin® concentration 5 µl/ml serum) was added to normal serum It was therefore concluded that the *broad* abnormal band was due to PVP

The stored material in liver bone marrow and ovary the staining properties characteristic of PVP (13) the treatment with Insipidin Retard was dis nued Repeated lipoprotein electrophoreses were d and six months later both abnormal bands had disappeared

At a control examination at this time serum alkaline phosphatase was still increased (6 78 µkat/l) while aspartate amino transferase was normal Repeated bone marrow and liver biopsy examinations (performed after the patient had given her informed consent) still showed storage of PVP judged to be of the same magnitude as previously This patient had received a total dose of approximately 900 g of PVP (1 ml Insipidin Retard contains 0 250 g PVP)

Due to the finding in this patient we examined three more patients who according to the hospital files were on long term treatment with Insipidin Retard

Case 2

A 64-year-old female who 11 years previously (in 1966) had suffered from arachnoiditis of the optic chiasm After a neurosurgical intervention for this condition in 1969 diabetes insipidus was detected and the patient was treated with Insipidin Retard On the present admission informed consent was given to bone marrow examination and liver biopsy both showed histological findings typical of PVP storage No inflammation or fibrosis in relation to the storage was found The lipoprotein electrophoretic pattern was identical to that seen in case 1 As the patient complained of urinary incontinence an i v pyelography was done which showed a tumour mass in the left kidney A nephrectomy was performed the tumour was a renal cell cancer of clear cell type Many groups of PVP containing macrophages were found inside the tumour (Fig 2) In areas of the kidney not involved by tumour PVP storage was seen in macrophages in the interstitial tissue but to a much lesser extent than in the tumour The patient had received approximately 450 g of PVP

Case 3

A 36-year old male treated for diabetes insipidus with Insipidin Retard since the age of 3 The patient had no complaints Informed consent for bone marrow examination was given The histological findings were typical of PVP storage No inflammation or fibrosis in relation to the storage was found The serum lipoprotein electrophoretic pattern was identical to that seen in case 1 The patient had received approximately 1 700 g of PVP

Case 4

A 23 year-old female treated for 14 years for diabetes insipidus with Insipidin Retard She had no complaints and did not consent to biopsy procedures The serum lipoprotein electrophoretic pattern was identical to that seen in case 1 and was normalized three months after withdrawal of Insipidin Retard The patient had received approximately 250 g of PVP



Fig 2 Case 2 Renal cell carcinoma Groups of macrophages with vacuolated cytoplasm containing PVP are seen within the tumour (arrows) Hematoxyl n-eosin ×276

DISCUSSION

PVP does not harm the human organism when given orally or applied to the skin as only insignificant amounts are absorbed (2 15) After parenteral administration low molecular forms of PVP (mol wt approximately <30 000) are cleared by the kidneys in a few days but with increasing molecular weight the fraction of retained PVP increases (10 12) leading to an accumulation of PVP in the tissues especially the reticuloendothelial system (8 10) Inflamed areas and malignant tumours have a particular affinity for PVP caused by the disturbance in vascular permeability and the activation of macrophages (8 10 15)

The question then arises whether this storage is harmful or not More than half a million people have received infusions with PVP as a plasma expander and there is no evidence that this short term application is dangerous (15) A smaller number of patients receive PVP daily for years in pharmaceutical preparations (as Insipidin Retard) Case histories showing a number of signs and symptoms in such patients have been published over the years Cabanne et al (3) related PVP storage to occurrence of pulmonary fibrosis artralgia and cutaneous nodules Couinaud et al (4) reported a case of PVP storage in omental fat causing intestinal obstruction Bert et al (1) described a case of pancytopenia and decreased renal function probably due to PVP storage in bone marrow and kidneys In Scandinavian literature Reske Nielsen et al (13) reported two patients with PVP storage one with subcutaneous nodules and the other with spontaneous fracture of left femoral bone and polyneuropathy PVP was found in the skin muscle and nerve tissue Edelmann et al (7) reported three patients with PVP storage one with PVP storages in omental fat *and retroperitoneal tissue causing obstruction of* the duodenum and ureters another with pulmonary fibrosis in whom a lung biopsy revealed PVP storage the third patient is case 1 in this paper Among our patients cases 3 and 4 were asymptomatic In case 2 a renal cell carcinoma was found but the patient had no symptoms that could be related to PVP storage Case 1 had polyneuropathy of unknown etiology secondary amenorrhoea and sterility A review of the muscle and nerve biopsy taken during neurological examination in 1969 did not reveal PVP deposits Storage was found in the ovary but on the other hand a contributory pituitary insufficiency was a possibility Storage was found in the liver too and this may be the cause of the slightly increased alkaline phosphatase level but one must also consider the possibility of a side-effect of anticonvulsant treatment (14)

Most authors believe that PVP is inert biologically and that no metabolism takes place (10 11 15) If this is true the in vivo presence of an abnormal band anodically to the application line on lipoprotein electrophoresis is not easily understood In our patients no histological evidence of tissue reaction against the storage could be demonstrated

Reviewing the literature Wessel et al (15) concluded that PVP has no carcinogenic effect since no cancer was found in patients receiving high molecular PVP for years Our case 2 represents such a patient in whom a cancer actually was found although no causal relationship can be postulated

The various signs and symptoms in patients with PVP storage are by no means conclusive

many of these states might be due to a causal coincidence of other diseases in patients thus treated However they leave a suspicion that long term treatment may be harmful Our case reports cannot weaken this suspicion

The presence of PVP in tissues has led to serious histologic misinterpretations Leder and Lennert (9) report a number of pitfalls caused by the presence of PVP in tissues ranging from tuberculosis to cancer From a clinical point of view the possibility of faulty diagnosis is equal ranging from pa raamyloidosis (5) to our suspected inborn error or metabolism in case 1 Unawareness of PVP storage may in this way represent a latent risk for the pa tient

Although no clear-cut etiological or pathogenic role of PVP can be stated we feel that a withdrawal of PVP-containing pharmaceuticals for long term parenteral treatment is advisable

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Verapamil and Pulmonary Hypertension

Knud Landmark Anne Marie Refsum Svein Simonsen and Ole Storstein

From Medical Department B Rikshospitalet University of Oslo Oslo Norway

ABSTRACT We report on the effect of verapamil in 12 patients suffering from pulmonary hypertension The drug caused a slight, but statistically significant decrease in mean pulmonary artery pressure and in the work performance by the right ventricle The mean pressure of the right atrium the end diastolic pressure of the right ventricle, the pulmonary arteriolar resistance, the cardiac index and the stroke volume were not significantly changed, however, and there was a wide spread of the values observed In some patients the drug exerted a marked negative inotropic effect, with a concomitant increase in the pulmonary arteriolar resistance

Verapamil blocks the calcium (Ca^{++}) influx in vascular smooth muscle and induces electromechanical decoupling (2 5 6) In animals the drug lowers coronary vascular resistance and increases myocardial blood flow in vitro (9) as well as in vivo (11 12) In 11 normal individuals Luebs et al (8) found that verapamil significantly increased coronary blood flow Verapamil also lowers systemic arterial blood pressure (1 4 7) the total peripheral resistance is unchanged (13) or slightly reduced (1) In dogs verapamil causes peripheral vasodilation (11) In patients with renal hypertension 5 mg verapamil i v caused the systolic and diastolic BP levels to fall by *approximately 25 and 22%* respectively within 1 min (4)

In isolated rat lungs verapamil inhibited the hypoxic pulmonary vasoconstriction (10) Haeusler (6) has shown that verapamil could competitively antagonize calcium induced contraction of strips of depolarized rabbit pulmonary arteries Several agents have been tried in the hope of finding a drug capable of reducing an elevated pulmonary artery pressure but so far the results have been disappointing (17) The present study was therefore performed to see if verapamil could lower the pulmonary artery pressure in patients suffering from pulmonary arterial hypertension

PATIENTS AND METHODS

Twelve patients were studied during diagnostic cardiac catheterization They were all in sinus rhythm Informed consent was obtained from all Their age diagnosis and physical characteristics are shown in Table I

The investigation was carried out in the morning in a fasting state and the patients stayed in a supine position throughout the study They were premedicated with 0 1 g allypropymal Any prior medication with digitalis and/or diuretics was continued A right cardiac catheterization was performed and the catheter (Cournand no 7 or 8) was introduced percutaneously through the right femoral vein and advanced to the right atrium The pressures in the right atrium right ventricle and pulmonary artery were recorded on a Mingograph 81 (Elema Schönander Stockholm Sweden) at the same time as the ECG In some patients the pulmonary capillary venous (PCV) pressure was recorded as well The pressures and the ECG were monitored simultaneously and continuously on an oscilloscope Cardiac output (Q) was estimated using the Fick principle Expired air was collected for 3 min and analyzed for O_2 and CO_2 content by Scholander apparatus Verapamil 0 15 mg/kg (total amount shown in Table I) was then slowly injected (1 mg/min) into the pulmonary artery A commercially available preparation of verapamil Isoptin® (Knoll AG) was used in the experiments Approximately 10 min after termination of the injection Q was re-estimated and the pressures were again recorded The following formulas were used in the calculation

1 Pulmonary arteriolar resistance (PAR)=

$$\frac{\text{mean pulmonary arteriolar pressure} - \text{mean PCV pressure}}{\dot{Q}} \times 80 \text{ dyn/sec cm}^{-5}$$

In most patients it was impossible to measure PCV pressure (Table II) and PAR was then calculated from an *estimated PCV pressure of 10 mmHg*

Abbreviations PCV=pulmonary capillary venous $\overline{PCV}$=mean PCV pressure PAR=pulmonary arteriolar resistance $\overline{PA}$=mean pulmonary arteriolar pressure *$\overline{RA}$=mean pressure of the right atrium WRV=work of* the right ventricle RVEDP=end-diastolic pressure of the right ventricle $\dot{Q}$=cardiac output (l/min) CI=cardiac index BP=mean systemic arterial pressure rate SV=stroke volume

Table I *Details of the patients*

Pat no	Age (y)	Sex	BSA (m^2)	Diagnosis	Digitalis	Total amount of verapamil (mg)
1	69	♂	1 77	Primary pulmonary hypertension	+	9 5
2	21	♂	1 19	Secondary pulmonary hypertension (ASD)	–	6 9
3	31	♀	1 65	Primary pulmonary hypertension	–	9 3
4	62	♂	1 92	Primary pulmonary hypertension	–	12 0
5	61	♂	2 06	Primary pulmonary hypertension	+	12 2
6	64	♂	1 73	Primary pulmonary hypertension	+	9 9
7	39	♀	1 57	Primary pulmonary hypertension	–	9 2
8	34	♂	1 88	Secondary pulmonary hypertension (VSD)	–	10 5
9	55	♀	1 84	Primary pulmonary hypertension	–	11 7
10	50	♂	1 90	Primary pulmonary hypertension	+	11 3
11	53	♂	1 48	Primary pulmonary hypertension	–	7 7
12	48	♀	1 35	Pulmonary fibrosis	+	5 0
Mean	49		1 70			9 59
±S D	14 8		0 26			2 19

2 Work of the right ventricle (WRV)=

$$\frac{\text{mean pulmonary arteriolar pressure} \times \dot{Q} \times 13\,6 \text{ kgm/min}}{1000}$$

The effect of verapamil was tested by means of Student s *t* test for paired observations

RESULTS

The mean pressure of the right atrium ($\overline{RA}$) was slightly elevated in some of the patients investigated (Table II) Verapamil caused a small statistically insignificant increase in the mean value of $\overline{RA}$ in one patient (no 10) $\overline{RA}$ however rose from 12 to 24 mmHg The end-diastolic pressure of the right ventricle (RVEDP) was slightly elevated in most patients before i v injection of verpamil the drug did not cause any further significant increase in the mean value The mean pulmonary artery pressure ($\overline{PA}$) was in every patient well above the expected upper normal limit (15) verapamil caused a slight but statistically significant decrease in the mean value (Table II) In the 4 patients in whom it was possible to obtain the mean PCV pressure ($\overline{PCV}$) the values were normal with one exception

II *Haemodynamic changes after verapamil*

efore A=after verapamil

Pat no	$\overline{RA}$ (mmHg)		RVEDP (mmHg)		$\overline{PA}$ (mmHg)		$\overline{PCV}$ (mmHg)		PAR (dyn/sec cm^{-5})		CI (l/min)		WRV (kgm/min)	
	B	A	B	A	B	A	B	A	B	A	B	A	B	A
1	4	7	7	14	45	42	9	7	236	283	6 9	5 6	7 46	5 63
2	5	1	10	10	80	68								
3	4	7	11	11	62	58			1 026	1 257	2 4	2 1	3 14	2 76
4	7	8	14	10	42	41	11	8	426	533	3 1	2 5	3 54	2 68
5					53	46			500	482	3 5	3 1	5 19	3 94
6					42	32			861	1 219	2 2	1 2	2 23	0 91
7					42	38	8	6	480	582	3 8	2 8	3 43	2 [illegible]
8	4	4	7	4	31	26			460	298	2 0	2 3	1 56	1 52
9					35	38			277	345	4 4	3 1	3 86	2 21
10	12	24	26	30	120	96			1 667	985	2 5	3 4	7 83	8 43
11	9	12	15	17	64	62			1 271	755	2 3	3 5	2 96	4 47
12	0	0	10	6	70	66	2	2	1 250	1 575	3 2	2 5	4 19	2 96
Mean	5 6	7 9	12 5	12 8	57 2	50 3	7 5	5 8	769	755	3 3	2 9	4 13	3 45
±S D	3 7	7 6	6 2	8 1	24 7	20 4	3 9	2 6	475	441	1 4	1 1	1 98	2 14
	0 20>p>0 10		p>0 5		p<0 01				0 50>p>0 10		0 20>p>0 10		p=0 05	

Verapamil slightly lowered $\overline{PCV}$ but the observa tions were too few to allow any statistical calcula tion Verapamil caused a slight statistically in significant decrease in PAR However there was a wide spread of the values and while PAR was markedly increased in one patient (no 6) it was re duced in others (nos 8 10 and 11)

While the values for cardiac index (CI) were within normal limits those for WRV were elevated in all patients (Table II) Verapamil caused a small insignificant decrease in the mean value for CI and a statistically significant decrease in the mean value for WRV However a wide spread was observed and in one patient (no 6) a marked drop was observed in CI and WRV and when the catheteriza tion was terminated he had a transitory drop in systemic arterial pressure associated with dys pnoea he recovered before any treatment was given Verapamil induced increases in CI and WRV were observed in 2 patients (nos 10 and 11)

While a decrease in $\overline{PA}$ was usually accompanied by a decrease in CI after verapamil the opposite relationship was observed in 3 patients (nos 8 10 11) In 4 patients in whom the mean systemic arter ial pressure (BP) was recorded before and after verapamil the drug caused a moderate reduction Heart rate (HR) and stroke volume (SV) were un changed (Table II) ECG was not influenced by verapamil except for a slight prolongation in the PQ interval

3P mmHg)		HR (beats/min)		SV (ml)	
3	A	B	A	B	A
20	99	55	51	222	198
		76	81	51	44
		58	58	107	83
		75	60	96	105
		80	85	49	25
		67	62	89	71
115	114	65	60	56	72
		78	77	104	75
134	111	78	77	62	87
100	94	71	70	48	76
		92	90	48	37
117	105	72	70	85	79
14 0	9 5	10 6	12 7	51	46
		$0\ 50 > p > 0\ 20$		$0\ 50 > p > 0\ 20$	

DISCUSSION

Intravenous injection of verapamil caused a slight but statistically significant decrease in the mean value of $\overline{PA}$ In patients with normal $\overline{PA}$ values Sloman et al (14) found no verapamil induced changes Ryden and Sætre (13) and Atterhog and Ekelund (1) on the other hand found a slight but statistically significant increase in $\overline{PA}$ and they suggested that this phenomenon could be accounted for either by an increased pulmonary resistance or a slightly reduced performance of the left ventricle with increased left atrial filling pressure However in four patients in whom we were able to record $\overline{PCV}$ normal values were found before and after verapamil Normal $\overline{PCV}$ values in patients with primary pulmonary hypertension have previously been recorded by Storstein et al (17)

Verapamil depresses contractile force in vitro (9 11) but in dogs receiving verapamil in a smaller dose HR Q and left ventricular work increased (12) In the animals which were pretreated with a β adrenergic receptor blocking agent and reserpine these effects were abolished—an observation which supports the hypothesis that catecholamines are re leased in response to verapamil (11) In patients with cardiac disease verapamil i v produced mod est increases in CI and left ventricle end diastolic pressure (13) Atterhog and Ekelund (1) noted on the other hand a mild negative inotropic effect of verapamil in 8 healthy middle aged men this effect was abolished by exercise Our results are on the whole in accordance with these observations as the mean values for the filling pressure of the right ventricle the RVEDP and CI did not change significantly However there was a wide spread of the values observed and in one patient verapamil caused a modest decrease in $\overline{PA}$ with a concomitant pronounced reduction in CI and WRV resulting in a substantial increase in PAR However in three pa tients a slight to modest fall in $\overline{PA}$ was accompanied by an increase in CI resulting in a marked decrease in PAR In two of these patients this brought about an increase in WRV This phenomenon might be explained by an increased filling pressure of the right ventricle (patients 10 and 11) (RVEDP and SV increased) and/or by a reflexly mediated liberation of catecholamines in connection with verapamil (12) From a haemodynamic point of view one should therefore be very cautious about tre patients suffering from pulmonary hy

with verapamil The drug may cause a drastic decrease in CI or a further increase in the strain on the right ventricle The values for WRV in this investigation are as expected well above those found in patients with normal $\overline{PA}$ values (18)

The main stimulus to anoxic pulmonary hypertension seems to be a reduction in alveolar O_2 tension (16) which initiates transmembrane calcium influx in smooth vascular muscle (3) It has been stated that the inhibition of hypoxic pulmonary vasoconstriction in isolated rat lungs by verapamil indicates that the hypoxic mechanism is critically dependent on the transmembrane influx of extracellular calcium (10) However the characteristic pathological changes observed in pulmonary arterial hypertension are pulmonary arteriosclerosis medial hypertrophy of the muscular arteries and arterioles and marked intimal proliferation, with narrowing of the arterial lumen and occasional complete occlusion of the lumen by organized thrombi (17) It is therefore unlikely that such irreversible pulmonary artery changes will respond to verapamil therapy to any greater extent

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Absorption and Side-Effects after Peroral Administration of Sustained Release Iron Tablets

Ferro-Retard® Compared with Ferronicum® and Duroferon Duretter®

F Levy P Andersen and T Ekren

From Deaconess Hospital Lovisenberg and the Hematological Research Laboratory Ullevål Hospital Oslo and Collett Marwell Hauge a/s Asker Norway

ABSTRACT *Study I* In 13 iron deficient patients the absorption from 100 mg ferrous sulphate (Ferro-Retard®) after 3 and 5 hours was comparable to the absorption from 176 mg ferrous gluconate in rapidly disintegrating tablets (Ferronicum®) *Study II* (*a*) The serum iron elevation in 14 blood donors with normal Hb after administration of ferrous sulphate (100 mg Fe^{++}) in Ferro-Retard or Duroferon Duretter® seemed to be equal when tested on the 4th day after donation of 450 ml blood (*b*) The iron absorption measured by the elevation of serum iron 5 and 8 hours after administration of sustained release tablets, proved to be better on the 11th and especially on the 16th day than on the 4th day after donation *Study III (side effects)* In a study on 113 subjects the total number of complaints was unusually high both before and after administration of placebo rapidly disintegrating and sustained release ferrous sulphate tablets The selection of participants and other methodological factors have probably been of importance Hence, comparisons between different studies of side-effects should always be carried out with caution

Dyspeptic side-effects of iron tablets may be caused by high local concentrations of ferrous ions in the gastric mucosa. Patients who experience such side-effects would probably tolerate medication with iron tablets which gradually release their contents in the stomach duodenum and jejunum better than with conventional preparations The gradually released ferrous ions from sustained release tablets during 6 hours are less affected by gastric contents e g the ferrous ion binding phytates than iron from rapidly disintegrating tablets (10) This should facilitate the availability of iron for absorption in the small intestine

The absorption of enterosoluble and sustained release tablets has been shown to vary widely from preparation to preparation (7 12) A new sustained release iron preparation Ferro-Retard Collett® containing 100 mg Fe^{2+} as sulphate has been introduced on the market The iron is gradually released from a biologically inert harpix resin In vitro about 40% is released during the first hour a maximum of 65% after the second hour and a minimum of 80% after 6 hours (1) With two daily doses this should provide enough iron for the erythropoiesis in patients with iron deficiency if adequately absorbed In iron deficient patients the iron absorption is increased (4 13 14 15) in the more distal as well as the proximal part of the jejunum (24)

This study in three parts aimed at comparing the absorption of Ferro-Retard® 100 mg Fe^{2+} as sulphate with that of Ferronicum Sandoz® 176 mg Fe^{2+} as gluconate (study I) and with Duroferon Duretter® 100 mg Fe^{++} as sulphate (study II) and finally to register side-effects compared with placebo and ferrous sulphate 100 mg Fe^{++} in rapidly dissolving tablets during controlled conditions (study III)

GENERAL METHODS

The test for iron absorption was performed according to the method of Jasinski (15 17) Blood was collected in the fasting state at 08 00 a m for determination of haemoglobin (Hb) serum iron and total iron-binding capacity (TIBC) (performed at the Central Laboratory Oslo City Hospital as part of the routine) Thereafter the iron dose was given and serum iron was determined after 1 3 5 and 8 hours

SUBJECTS AND METHODS

Study I Comparison between iron absorption from Ferro-Retard 100 mg Fe^{2+} and Ferronicum 176 mg Fe^{2+}

The iron absorption was tested in 13 patients aged 35-84 years in the general medical ward at Krohgstøtten Hospital Oslo The clinical diagnosis was iron deficiency with

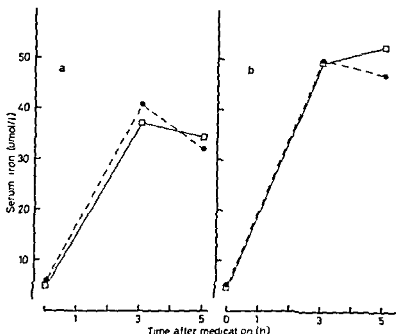


Fig 1 Iron absorption curves after administration of Ferronicum Sandoz 176 mg Fe^{2+} (●---●) and Ferro-Retard Collett 100 mg Fe^{2} (□—□) Mean values for all 13 patients (a) and for 6 patients with iron deficiency anaemia (b)

Hb values of 70–126 g/l and TIBC saturation less than 16% (2 13) In addition to iron deficiency seven patients had other disorders known to affect the iron absorption (6) pneumonia arthritis urica arteritis temporalis cardiac insufficiency and malabsorption Four females (nos 1 4 5 11) and two males (nos 6 9) had uncomplicated posthaemorrhagic iron deficiency anaemia and were evaluated separately and compared with the total heterogeneous group of patients

The test was performed as a cross-over trial between Ferronicum 8 tablets = 176 mg Fe^{2} and Ferro-Retard 1 tablet = 100 mg Fe^{2} given in the morning after an over ...ht fast Serum iron was determined after 0 3 and 5 ... The second preparation was administered 3–7 days ...r the first dose

Study II Comparison between absorption of Ferro-Retard and Duroferon Duretter in blood donors after blood donation

Fourteen healthy blood donors aged 20–63 years (9 males and 5 females) were analyzed on day 0 for serum iron TIBC and Hb before donating 450 ml blood at Blodbanken Oslo City Hospital Thereafter they were kept on an iron deficient diet which was standardized with regard to contents and timing on the test days

On the first test day day 4 serum iron TIBC and Hb were determined before administration of one tablet of either Ferro-Retard 100 mg Fe^{2+} (preparation A) or Duroferon Duretter 100 mg Fe^{2} (preparation B) Serum iron was determined after 1 3 5 and 8 hours On day 11 the two preparations were crossed over in 10 subjects whereas 4 subjects received preparation B the second time On day 16 these 4 subjects took preparation A The preparations were administered blindly Complaints of side-effects were registered

Study III Side effects

To examine the side-effects of Ferro-Retard a multicenter trial involving 113 healthy adults (59 females and 54 males) aged 19–55 years was carried out Included were 58 students and 13 employees at the Dental Faculty University of Oslo 11 students and 7 employees at Oslo City Hospital Medical Department IX and 24 laboratory employees at the Deaconess Hospital Lovisenberg Oslo

The preparations administered were placebo (A) containing 0 mg Fe^{2} traces of FeO in the coating material—rapidly disintegrating $FeSO_4$ (B) tablets 100 mg Fe^{2+}—and Ferro-Retard (C) sustained release $FeSO_4$ 100 mg Fe^{2} All the preparations were produced in the Laboratory of Collett Marwell Hauge a/s Asker for a double blind trial B disintegrated after 7 min in H_2O at 37 C Both A and B fulfilled the requirements of interdose variations and content uniformity set out by Ph Nord III and USP XVIII respectively (1)

The trial period for each person lasted for 30 days divided into 3 intervals of 10 days with different iron medication or placebo

Each of the 5 groups of participants was stratified according to sex and divided into three subgroups Each of them received the iron preparations in the order ABC BCA or CBA on day 0 10 and 20 together with a questionnaire and instructions for taking 1 tablet in the morning and in the evening The contents of the bottles were 21 (A) 22 (B) or 23 (C) tablets and information on the number of remaining tablets was requested on the questionnaires collected on days 10 20 and 30 In addition to a reminder to swallow the tablets whole the subjects were asked to cross for the following side-effects 1) Heartburn or belches 2) Pain in the epigastrium 3) Nausea and/or vomiting tendency 4) Abdominal distension (borborygmia and meteorism) 5) Diarrhoea 6) Constipation They were also asked to record any of the above symptoms exten-

Table I Iron absorption data after administration of Ferronicum Sandoz 176 mg Fe (S) and Ferro-Retard Collett 100 mg Fe^{2} (C) in study I

Pat no	Age (y)	Sex	Test interval (d)	Hb (g/l)	TIBC (μmol/l)	Serum iron (μmol/l) after iron administration 0 h	3 h	5 h	Preparation
1	52	♀	3	103	61	7.0	37.1	28.5	S
				109	65	6.4	54.2	54.4	C
2	43	♂	3	83	73	3.4	5.2	3.4	S
				86	78	3.8	23.1	21.8	C
3	34	♀	3	107	84	9.5	55.8		S
				110	78	4.7	19.5	10.6	C
4	65	♀	3	70	84	6.8	46.9	36.0	S
				70	83	2.7	37.4	58.7	C
5	49	♀	4	76		3.8	44.2	48.0	S
				71	63	3.2	61.2	60.1	C
6	76	♂	6	80	74	4.3	49.8	48.0	S
				76	75	5.5	27.9	25.6	C
7	59	♂	4	105	62	5.7	20.6	12.0	S
				105	64	5.5	46.0		C
8	61	♂	4	109	71	5.9	26.5	15.2	S
				104	71	4.3	19.3	12.4	C
9	67	♂	4	85	75	3.6	67.8	56.9	S
				88	78	6.3	53.9	50.1	C
10	62	♂	7	100	71	4.3	16.5	8.4	S
				100	71	5.7	30.1	6.9	C
11	84	♀	4	85	73	3.8	52.4	64.8	S
				88	73	4.3	62.8	67.8	C
12	72	♂	7	1[illegible]6	62	12.4	51.9		S
				126	69	2.9	25.4	11.8	C
13	74	♀	6	86	58	5.5	55.7		S
				87	63	5.2	21.3	14.0	C
Mean ± S.D.			4.5±1.5	93.5±16.1	70.7±8.5	5.8±2.6	40.8±18.4	32.1±21.8[b]	S
				93.9±16.8	72.8±6.5	4.7±1.2	37.1±16.4	34.5±21.9	C

[a] n = 12 [b] n = 10

enced before the first medication and were told to stop medication if unpleasant side-effects occurred.

Statistics

The data were evaluated statistically by a CDC 3300 at the University of Oslo by Ø. Hasvold. The F test was used to exclude variances between groups, and Student's t test was used to evaluate differences between the various preparations. The Mann Whitney U test and χ^2 test were used for paired comparisons between the separate iron medication groups in study III.

RESULTS

Study I

The data obtained for the 13 patients are presented in Table I. Fig. 1 shows their mean values for serum iron compared with the extracted values for the 6 patients with uncomplicated iron deficiency anaemia. The higher serum iron levels in iron deficient patients, indicating better absorption, are apparent and in agreement with previous reports (13, 14, 15, 17).

There was no significant difference between serum iron concentrations after administration of 100 mg Fe^{2} in Ferro-Retard sustained release preparation and after the higher dose of 176 mg Fe^{2} in rapidly disintegrating Ferronicum tablets. With Ferronicum, maximum serum iron concentrations were reached after about 3 hours in iron deficient patients, but seemed to be slightly higher with Ferro-Retard after 5 hours, indicating prolonged absorption with the sustained release preparation. However, the differences were not [illegible]ficant.

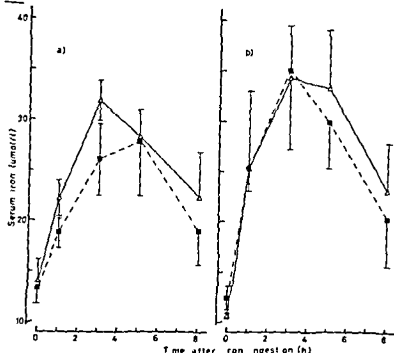


Fig 2 Increase in serum iron concentrations (mean±S E M) after administration of 100 mg Fe² in Ferro-Retard Collett (Δ—Δ) and Duroferon Duretter (■- - -■) Samples drawn 4 days after blood donation from 7 healthy subjects on each preparation *(a)* and *11* days after blood donation from 11 healthy subjects on Duroferon Duretter and from 3 on Ferro-Retard *(b)*

Study II

Table II shows the relevant data for the two groups compared and that they are evenly distributed The serum iron values obtained on days 4 11 and 16 after blood donation are set out in Table III When the data were treated statistically it appeared from the initial serum iron concentrations as well as from the TIBC concentrations that the subjects probably exhibited different stages of iron absorption on the e test days As a result of this finding combined h the unbalanced distribution of preparations administered the data from each test day were analyzed separately

Fig 2*a* shows the variation of serum iron on day 4 after 100 mg Fe^{2+} in 7 subjects on preparation A and in 7 subjects on preparation B Fig 2*b* shows the variation on day 11 for 3 subjects on preparation A and 11 subjects on preparation B Both preparations caused a significant increase in serum iron The intercurve profiles are not statistically different on either of the two test days The maximum serum iron levels are somewhat higher on day 11 than on

Table II *Personal data and haematological values on the day of blood donation in study II*

Subj no	Age (y)	Sex	Hb (g/l)	Serum iron (µmol/l)	TIBC (µmol/l)	TIBC saturation (%)
1	33	♂	168	14 1	63 4	22 3
2	21	♂	163	24 3	65 0	37 5
3	34	♂	156	22 9	54 2	42 2
4	28	♀	130	13 8	45 6	30 2
5	28	♂	156	22 6	66 6	33 9
6	58	♂	153	13 2	65 5	20 2
7	24	♀	145	9 3	56 4	16 5
8	32	♀	148	12 2	52 6	23 1
9	63	♂	157	17 5	56 9	30 8
10	23	♂	155	8 4	67 7	12 4
11	30	♂	150	18 8	63 9	29 9
12	21	♂	143	29 5	62 3	47 4
13	20	♀	154	12 5	59 1	21 2
14	21	♀	148	20 6	46 2	44 6

Table III *Iron absorption data after administration of 100 mg Fe^{2} from either Ferro Retard (preparation A) or Duroferon Duretter (preparation B) in study II*

Subj no	Preparation	Serum iron (μmol/l) after iron administration					TIBC (μmol/l)	Hb (g/l)
		0 h	1 h	3 h	5 h	8 h		
1	A	13 2	16 8	24 3	22 2	15 8	71 1	155
	B	17 9	26 5	31 7	27 9	18 3	69 6	157
2	A	21 1	24 0	35 8	39 9	44 4	65 0	162
	B	14 5	27 6	38 3	33 1	21 7	64 1	159
3	A	9 3	17 9	29 7	23 1	20 2	54 2	146
	B	12 9	34 2	51 4	48 7	34 5	68 0	155
4	A	20 4	28 3	37 6	30 8	20 4	41 2	136
	B	11 6	15 2	20 6	17 0	5 9	49 6	130
5	A	17 5	26 9	36 9	34 5	29 2	70 0	155
	B	6 3	12 0	13 3	7 9	2 9	63 9	154
6	A	6 8	23 6	33 1	26 9	14 9	67 1	145
	B	9 8	31 5	49 8	39 7	30 1	68 2	141
7	A	9 5	17 9	26 1	20 8	12 2	70 3	140
	B	16 8	24 7	31 3	25 9	13 1	56 9	134
8	B	7 2	12 5	21 3	22 6	17 0	52 1	141
	B	14 0	25 8	38 7	28 5	15 8	59 4	140
	A	7 2	26 7	28 5	–	49 4	82 1	145
9	B	10 2	23 3	11 1	9 1	7 3	60 1	152
	B	14 5	22 2	22 9	18 6	8 6	60 1	151
	A	23 5	34 4	–	31 0	26 1	69 8	155
10	B	17 2	14 9	40 1	49 2	–	71 1	143
	B	5 7	22 5	24 5	21 1	14 7	77 9	149
	A	8 8	27 6	–	43 3	55 1	79 5	146
11	B	10 6	22 2	36 2	30 1	16 6	69 8	147
	B	12 5	36 0	63 7	63 5	59 2	77 9	143
	A	18 3	37 2	57 1	57 1	55 4	79 5	152
12	B	17 0	18 6	23 4	21 8	18 6	47 1	135
	A	10 2	16 6	20 6	22 0	13 8	70 9	142
13	B	17 0	19 3	24 9	34 5	32 6	63 9	122
	A	10 9	19 3	37 4	37 6	29 9	65 5	135
14	B	14 0	21 1	25 6	–	21 7	47 8	131
	A	10 7	40 5	45 1	40 8	26 0	54 8	142

day 4 after blood donation but this difference is not statistically significant either

Subjects 8 9 10 and 11 received preparation B on days 4 and 11 and preparation A on day 16 The serum iron values on the three test days for this group are profiled in Fig 3 The increasingly better absorption of iron over the period 4–11–16 days after blood donation is demonstrated All the levels of serum iron as well as TIBC saturation percentages are significantly higher on day 16 than on day 4 ($p<0.05$) Especially the 8 hour values after Ferro-Retard are much higher than on the two preceding test days

The number of side effects registered was minimal Only one subject had some feeling of abdominal distension and rumbling after both preparations He was predisposed to this having an irritable colon syndrome One subject with habitual constipation had possibly more tendency to constipation after Ferro Retard None of the participants had nausea epigastric pain or diarrhoea

Study III

Table IV lists the side effects reported before and after each iron/placebo medication The complaints against placebo were on the pretreatment level whereas the percentages of complaints after iron medication were significantly higher than after placebo

No clear difference in the total number of side

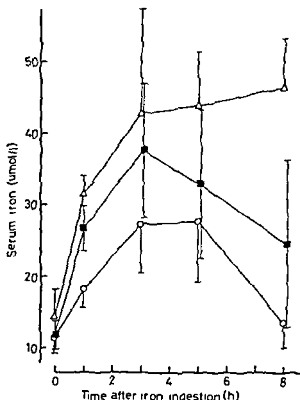


Fig 3 Increase in serum iron concentrations in 4 healthy subjects (mean±S E M) after 100 mg ferrous ions in sustained release $FeSO_4$ tablets 4 days (Duroferon Duretter ○—○) 11 days (Duroferon Duretter ■—■) and 16 days (Ferro-Retard Δ—Δ) after blood donation

effects between rapidly disintegrating and sustained ease $FeSO_4$ tablets (57 and 46% respectively) ld be detected with the statistical tests used e individual complaints of epigastric pain abdominal rumbling and distension and constipation were slightly more common after the rapidly disintegrating $FeSO_4$ tablets whereas diarrhoea developed slightly more frequently after sustained release tablets None of the differences however was statistically significant

The volunteers who discontinued medication did so during the first five days of the ten-day period The main reasons reported were epigastric pain and nausea after intake of rapidly disintegrating $FeSO_4$ tablets nausea and diarrhoea after Ferro-Retard and diarrhoea after placebo

The mean number of tablet taking days was of the same magnitude 9 8 on placebo and 9 6 on both rapidly disintegrating $FeSO_4$ and Ferro-Retard calculated from the remaining tablets after each test period

DISCUSSION

In a patient group with iron deficiency and infections a new sustained release tablet Ferro-Retard Collett was compared with Ferronicum Sandoz by postabsorption serum iron elevation In vitro experiments showed an almost complete release of the 176 mg Fe^{+} in Ferronicum after 7 min whereas only about 40% of the 100 mg Fe^{2+} in Ferro-Retard were released within 1 hour (1) In vivo however the serum iron elevation profiles after Ferronicum or Ferro Retard were about the same Compared with the rapid release of iron in the stomach from ferrous gluconate in Ferronicum the gradual release of ferrous ions from ferrous sulphate in Ferro-Retard might facilitate an efficient absorption through the distal part of the jejunal mucosa (24)

Table IV *Registration of side effects in study III*

	Before investigation	Placebo	100 mg Fe^{2} Rapidly disintegrating $FeSO_4$	Ferro-Retard
Total no of replies	113	107	107	107
Heartburn/belches	6	3	7	7
Pain in the epigastrium	2	4	13	9
Nausea/vomiting	0	0	8	8
Abdominal distension (borborygmia and meteorism)	13	9	24	18
Diarrhoea	1	5	12	15
Constipation	6	13	27	22
Cessation of medication	–	1	9	8
Subjects with one or more complaints (%)	17	22	57	46
Subjects who discontinued medication (%)	–	0 9	8 4	7.5

Our results also indicated a better absorption of both iron preparations in a group of patients with uncomplicated iron deficiency anaemia compared with that in patients with a combination of moderate iron deficiency anaemia and other intercurrent diseases (Fig 1)

Significantly higher maximum serum iron concentrations were observed in the iron deficient anaemic patients in study I (52 8 μmol/l) than in healthy blood donors (study II) with normal Hb (31 9 μmol/l) These observations are in accordance with previous reports (4 16 21)

In addition to the differences in the degree of absorption the iron elevation profiles varied Accordingly after administration of Ferro Retard we observed maximum serum iron levels after 3 hours in normal subjects (Fig 2*a*) after 5 hours in iron deficient patients (Fig 1*b*) and after 8 hours in blood donors when on day 16 iron deficiency with simultaneously increased erythropoiesis seemed to occur (Fig 3) (16 20)

In spite of large interindividual variations in the absorption of iron Ekenved (9) and Ekenved et al (11) found that the response on the serum iron level correlated well with total iron absorption and should therefore be useful in comparative studies of different iron preparations given to the same patient group by cross over technique This technique was used in study II No significant differences in iron absorption from Ferro-Retard and Duroferon Duretter were observed in healthy volunteers with small iron depots (blood donors) This in vivo bioavailability correlates well with the in vitro release data (1) It should however be stressed that without a difference of at least 50% in the absorption no qualitative differences between the two iron preparations can be expected with such a small number of participants (8)

Healthy blood donors without intestinal malabsorption are suitable participants in iron absorption tests as their iron depots are depleted after repetitive blood donations often followed by inadequate iron supplies After blood donation an increase in the erythropoiesis and a decrease in the iron depots leading to a better absorption (16 19) were to be expected and the serum iron elevation was indeed found to be higher on day 11 than on day 4

Interestingly the four subjects who received Duroferon Duretter on days 4 and 11 and Ferro-Retard on day 16 responded with a remarkably higher serum iron level on day 16 especially 8 hours after the intake of iron (Fig 3) An interpretation may be that at this time the erythropoiesis increased with a concomitant depletion of the iron reservoirs (20 21) and the changes in the jejunal mucosa leading to a better absorption of iron may need a period of at least 2½–3 weeks before maximal conditions for the absorption can be achieved Under these conditions a sustained release iron preparation may therefore be the better choice

Different methods of questioning often make it very difficult to compare side-effects of iron medication between one study and another In the present trial (study III) the participants were specifically asked to register side effects of different kinds and they received the questionnaire prior to testing The incidence of placebo responders was 22% which is only slightly higher than the pretreatment level (Table IV) Side-effects occurred at a frequency of 57% when using rapidly disintegrating ferrous sulphate tablets (100 mg Fe^{2+}) In a similar study (23) the percentages were 19 5 and 29 8 respectively with placebo and rapidly disintegrating ferrous sulphate tablets Thus even if the number of placebo responders with all its implications (3) were of the same magnitude in both studies nearly twice the participants registered side-effects in our study with rapidly disintegrating iron tablets The stated differences in questioning may be one of the reasons for this discrepancy Other factors may be found in the differences of the trial design Cross over technique was used in our study whereas a group comparative trial was performed in the other study referred to The participants in our study were also told to take the tablets on an empty stomach which probably may have increased the incidence of side-effects

After Ferro-Retard side effects occurred in 46% of the total number of replies Thus the ratio of side-effects compared with conventional iron tablets (46/157=0 8 Table IV) was of the same magnitude as after Duroferon Duretter (26 2/29 8=0 9) in blood donors However in pregnant women the reduction of side-effects after Duroferon Duretter was higher (23)

It is of importance during therapy in the clinical situation that the patients do not stop their medication In the present study the percentages of subjects who discontinued the iron medication (Table IV) were significantly lower than reported from corresponding groups (23) on placebo

rapidly disintegrating $FeSO_4$ (17 1%) and Durofe ron Duretter (13 4%) The side-effects occurring in the present study were thus probably less severe

Moreover the high incidence of side-effects in the present study does not correlate well with the clinical experience of medication with Ferro Retard as few patients on Ferro-Retard complained about side-effects as was also recently reported in teenagers on a dose of 100 mg ×2 for 60 days (18)

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Lidocaine and the Quarternary Ammonium Compound QX-572 in Acute Myocardial Infarction

A Comparative Study

Björn Bergdahl Erling Karlsson Jan Olof Magnusson and Christine Sonnhag

From the Department of Internal Medicine Division of Cardiology University Hospital Linköping Sweden

ABSTRACT Patients with suspected or proven acute myocardial infarction complicated by ventricular arrhythmias not corrected by lidocaine therapy (bolus dose 100 mg followed by infusion 2 mg/min) were treated either with an increased dose of lidocaine (bolus dose 50 mg followed by infusion 3 mg/min) or with 600 mg N N bis dimethylammonium chloride (QX 572 Astra Sweden) as an i v infusion during 30 min (3 patients) or 60 min (13 patients) In the lidocaine group the arrhythmias were controlled in 6 out of 15 patients, in the QX 572 group in 12 out of 16 a difference that is not statistically significant However, the frequency of side effects was significantly higher ($p<0\ 001$) in the QX 572 group (15 out of 16 patients) than in the lidocaine group (4 out of 15 patients) They were also more severe including pronounced tachycardia and hypertension It is concluded that despite the high antiarrhythmic effect of QX 572 an increase of the lidocaine dose would be safer and preferable in the clinical situation studied

Key words N N bis (phenylcarbamoylmethyl) dimethylammonium chloride lidocaine ventricular arrhythmias acute myocardial infarction
Acta Med Scand 204 311 1978

Lidocaine has become the drug of choice in the treatment of ventricular arrhythmias complicating acute myocardial infarction (AMI) However the drug has been reported to fail to terminate these arrhythmias in rather many cases (1 2 3 7 8 15) In part the failure was due to inadequate plasma concentrations using the routine dosage of 2 mg/min Higher doses of lidocaine seemed to be more effective but they may also increase the frequency of side-effects (1 15 24) Therefore it would be of interest to study the effect of other antiarrhythmic drugs in patients with arrhythmias which do not respond to conventional doses of lidocaine

The quarternary ammonium compound N N bis (phenylcarbamoylmethyl) dimethylammonium chloride (QX 572 Astra Sweden) has been shown to prevent and abolish ventricular arrhythmias in animals (4 5 12 17 25) and man (11 22 23) After an uncontrolled study Rydén et al (23) reported the drug to be very effective in patients with ventricular arrhythmias refractory to other treatments However no controlled study with comparison of both therapeutic effects and adverse reactions of QX 572 with those of lidocaine has been reported Therefore the present study was undertaken to investigate if patients with suspected or proven AMI and ventricular arrhythmias not responding to lidocaine 2 mg/min could be better treated by increasing the dose of lidocaine or with QX 572

PATIENTS AND METHODS

Patients admitted to the Coronary Care Unit (CCU) with suspected or proven AMI and exhibiting ventricular arrhythmias according to the criteria set by Lown et al (16) were treated with lidocaine (Xylocard® Hassle Sweden) i e a bolus dose of 100 mg followed by an i v infusion of 2 mg/min Included in the trial were those patients in whom the ventricular arrhythmias had not been controlled or recurred 0 5–24 hours after the initiation of lidocaine treatment Patients having a heart rate of <60 beats/min (BPM) second or third degree atrioventricular block atrial flutter or fibrillation manifest left heart failure systolic blood pressure (BP) <90 mmHg or a clinical state of shock were excluded The experimental procedure was fully explained to the patients and verbal consent was obtained The patients were allocated randomly to one of the following treatment groups

Lidocaine group An i v bolus dose of 50 mg lidocaine was given and the infusion rate of lidocaine was increased to 3 mg/min

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Abbreviations AMI=acute myocardial infarction CCU=Coronary Care Unit BPM=beats/min BP=b... sure ECG=electrocardiogram

Table I *Characteristics of the patients clinical and laboratory findings in the lidocaine and QX 572 groups*

	Lidocaine	QX 572
No of pats	15	16
Males	13	15
Females	2	1
Age (y)		
40–49	1	1
50–59	5	5
60–69	8	7
70–79	1	3
Mean	60 3	63 4
Previous AMI		
0	13	9
1	2	6
≥2		1
Verified AMI	13	13
Site of AMI		
Anterior	5	3
Lateral		1
Posterior	6	4
Uncertain	2	5
S ASAT max (μkat/l) (normal ≤0 70)		
<0 70	2	4
0 70–2 10	5	3
2 10–4 20	3	3
≥4 20	4	4
Mean	2 79	2 77
On digitalis on the day of study	1	5
Heart volume at discharge (ml/m² BSA)		
<500	9	5
500–600	3	5
700	1	1
700		3
missing	2	2

QX 572 group Lidocaine infusion was replaced by QX 572 using an infusion pump According to the recommendations of Rydén et al (22) all patients received a standard dose 600 mg of the drug was given in 200 ml saline The infusion time was 30 min in the first 3 patients and was then because of side-effects increased to 60 min

No further antiarrhythmic drugs or i v diuretics were administered The treatment was discontinued if serious side-effects developed Otherwise the patients were treated according to the routine of the CCU oxygen 4 l/min was usually given via a nasal catheter and hydromorphone or pentazocine was given i v for relief of pain The patients were allowed to drink and to eat light meals

Electrocardiogram (ECG) was continuously recorded on an 8-channel ink jet recorder (Mingograph 81 Siemens Elema) at a paper speed of 10 mm/sec All ECGs were analysed minute by minute according to Mogensen (18)

Table II *Number of patients exhibiting ventricular premature contractions (VPC) when entering the study and at therapeutic failure during treatment with lidocaine or QX 572*

VT = ventricular tachycardia

	Lidocaine		QX 572	
	At entry	At failure	At entry	At failure
Type of VPC				
>5/min	6	2	10	4
Multifocal	4	3	3	1
Paired	3	3	5	2
R on T	0	0	2	0
VT	7	2	2	0
No of pats	15	7	16	4

The ECG was also monitored by an oscilloscope Antiarrhythmic effects of the drugs were studied 60–210 min after start of therapy Therapeutic failure was defined as a recurrence of ventricular arrhythmias according to the criteria for entry to the study

BP was measured by cuff before and then every 10 min during the first hour of the study and then half hourly Significant changes in BP or heart rate (HR) were defined as follows *BP rise* Increase in systolic BP of ≥30 mmHg or in diastolic BP of ≥15 mmHg *BP fall* Decrease in systolic BP of ≥30 mmHg or to ≤90 mmHg *Increased HR* ≥20 BPM *Decreased HR* ≥20 BPM or to <60 BPM

Venous blood samples for assay of the plasma drug concentrations were taken from the contralateral arm to that used for the drug infusion before and at 15 30 60 and 210 min after the change in therapy Lidocaine concentrations were also measured in patients treated with QX 572 The plasma samples were frozen immediately and kept at −18°C until analysed Plasma lidocaine concentration was determined by gas liquid chromatography with flame ionization (14) carbocaine was used as the internal standard QX 572 in plasma was assayed by ion pair partition chromatography (20)

Differences between the two experimental groups were tested by Fisher s exact test or by Student s *t* test The level of significance was set to 1%

RESULTS

Table I presents some clinical details of the patients As judged from routine laboratory tests all had about normal renal and hepatic function and serum electrolytes were within normal limits

Plasma levels

The plasma levels of lidocaine at the moment of entry into the study were similar in the lidocaine and QX 572 groups (2 3±0 9 and 2 5±1 6 μg/ml respectively mean ± S D)

Table III *Type and frequency of side effects recorded 0–210 min after initiation of therapy with lidocaine (3 mg/min) or QX 572 (600 mg/30 or 60 min)*

	Lidocaine	QX 572
Total no of pats	15	16
Side-effects	4	15***
Fall in BP	4	5
Rise in BP	0	5[b]
Tachycardia	0	8**
Bradycardia	0	1
Emesis nausea	1	7
Circumoral paraesthesia	0	7**
Vertigo	1	0

[a] Three of the patients received QX 572 600 mg/30 min
[b] All 5 had tachycardia as well
*$p<0\ 01$ **$p<0\ 001$

In the *lidocaine group* the plasma concentrations reached 2 9±0 8 3 1±0 9 and 3 8±1 1 μg/ml at 15 60 and 210 min respectively after the increase of the dose The plasma levels of QX 572 in the *QX 572 group* were at corresponding moments 4 4±1 1 6 1±1 3 (end of infusion $n=13$) and 0 6±0 3 μg/ml The plasma lidocaine level in this group declined to 1 3±0 9 μg/ml at 60 min after the discontinuation and further to 0 9±0 7 μg/ml at 210 min

Therapeutic effects

The arrhythmias at the entry to the study and at therapeutic failure are listed in Table II

The drug administration was completed in 11 of the 15 patients in the *lidocaine group* Six of them were successfully treated without side effects In 5 patients arrhythmias recurred after an average of 92 min (range 60–139) The mean plasma lidocaine concentration at therapeutic failure in these 5 patients was similar to that at the end of the study in the 6 successfully treated patients (3 6 and 3 4 μg/ml respectively)

The first 3 patients in the *QX 572 group* were given this drug at the rapid infusion rate and in 2 of them the arrhythmias were abolished In the next 13 patients the infusion time was extended to 60 min and the arrhythmias were controlled in 10 of them The plasma concentrations of both lidocaine and QX 572 were also similar in this group in patients with and without therapeutic failure The difference in therapeutic efficacy between lidocaine and QX 572 was not statistically significant

Side-effects

There was a high frequency of side effects in both groups (Table III) Four of the 15 patients in the *lidocaine group* developed a mean BP fall of 1 30 mmHg after 10–95 min of infusion with the high lidocaine dose After discontinuation of the ... sion the BP in all patients returned to control within 15–30 min At the moment of BP fa... plasma levels of lidocaine ranged from ... to ... μg/ml which did not differ significantl... o... levels in patients without side-effects

In the *QX 572 group* the first 3 patie... received the drug at the rapid infusion rate ... developed a pronounced BP fall (mean 50/22 mm... The infusion had to be discontinued in thes... pa tients after 16 (therapeutic failure) 25 and 28 min After extension of the infusion time only 2 of the next 13 patients developed a BP fall of the same magnitude during the drug infusion At an average of 100 min (range 10–120) after the initiation of QX 572 therapy 8 patients exhibited tachycardia with a mean rise of HR of 33 BPM (range 26–40) Five of them also showed an associated rise of BP (mean 29/27 mmHg range 5–40/15–40) These haemodynamic changes mostly persisted for 3–5 hours Minor side effects i e circumoral paraesthesia emesis etc were recorded in 7 patients during the drug infusion The plasma concentrations of QX 572 and lidocaine were somewhat but not significantly higher in patients who showed haemodynamic side effects

DISCUSSION

Our results verify those of Ryden et al (22) that QX 572 is an effective antiarrhythmic agent However in the limited number of patients studied we found no significant difference to lidocaine in this respect

The major problem during our comparative study was the adverse effects of QX 572 This drug has previously been found to produce an initial vasodilatation and also an increased sympathetic tone (25) The early BP fall and the later tachycardia and hypertension recorded in this study probably reflect these pharmacological effects They seemed to be more pronounced and frequent than reported previously using the same or even higher doses of QX 572 (13 21 22 23) It should also be noted that late haemodynamic effects in our pat... started after the completion of drug

individualization of the dose as a means of reducing their frequency is hardly possible No established treatment exists for these late adverse effects Theoretically β adrenoceptor blockade would be effective but such a combined drug administration might also be risky

Modern trends in the treatment of AMI emphasize the importance of active reduction of heart work (9, 19) An unpredictable tachycardia and hypertension as now found after administration of QX 572 should therefore be avoided and in fact we found it necessary from ethical point of view to discontinue the investigation Thus it seems as if ventricular arrhythmia during routine lidocaine administration (2 mg/min) is treated more safely with an increased lidocaine dose than with QX 572

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Single and Multiple Beam Echocardiography in Aortic Valve Endocarditis

Report of Three Cases

Jan Fog Pedersen Jens Berning and Stig Haunsø

From The Ultrasonic Laboratory and the Cardiac Laboratory Gentofte Hospital Hellerup Denmark

ABSTRACT Three patients with aortic valve endocarditis were studied The single beam M mode echocardiographic findings comprised the appearance in diastole of a cluster of shaggy echoes at the aortic valve in all three patients Mitral flutter was seen in two patients and premature closure of the mitral valve in one patient At multiple beam two-dimensional echocardiography, the echo cluster could in all three patients be seen to move perpendicular to the sound beams, ascending into the aorta in systole and descending in diastole At valve replacement vegetations were found that explained the abnormal echo cluster The multiple beam echocardiography facilitated the interpretation of the single beam findings and increased the confidence therein By applying the non invasive modality of echocardiography in these patients with their high risk of embolism cardiac catheterization may possibly be avoided

Patients with infective endocarditis often present a difficult diagnostic problem particularly when blood cultures are negative (9) Direct evidence of the pathognomonic vegetations was provided until recently only by operation or autopsy However it has been shown that valvular vegetations may be demonstrated by echocardiography (3 11)

During the last few years conventional echocardiography has been extended by dynamic cross-sectional two-dimensional imaging of the heart Dynamic scanners are either mechanical with a fast moving ultrasound transducer (7) or multitransducer scanners wherein the sound beam is moved electronically (1 15) Both types of scanners have been used for several applications and the first reports on two-dimensional echocardiographic imaging of endocarditis vegetations have recently appeared (6 10)

We are reporting the single and multiple beam echocardiographic findings in three patients with surgically confirmed aortic valve vegetations

METHODS AND PATIENTS

Conventional single beam echocardiography was performed with a Smith Kline Instruments Echoline 20 ultrasound apparatus TM recordings were obtained from a Tektronix 564A oscilloscope on Polaroid films using open-shutter technique A complete recording of the left side of the heart was performed using the standard procedure (4)

Multiple beam cross-sectional cardiac imaging was performed with a locally constructed system containing an array of 30 transducer units activated sequentially (12) With a frame rate of 50/sec the system yields real time cross sectional images consisting of 30 parallel lines over an area of 65×140 mm The transducer array was placed obliquely in the precordium with the upper end at the left sternal border for longitudinal sections of the left heart and normal to this plane for transverse sections The echocardiographic examinations to be reported comprised both techniques and were performed less than 48 hours before surgery Clinical data on the patients are listed in Table I

RESULTS

The findings at conventional echocardiography are listed in Table II and typical examples are shown in Figs 1 and 2

The multiple beam cardiac image exhibited in all three patients a cluster of echoes in the aortic root moving abruptly proximally in systole and distally in diastole Frames from one examination are shown in Fig 3 The frames also illustrate the sequential movements of the aortic and mitral valves Furthermore the examinations in all three patients were suggestive of increased movements of the left ventricle secondary to volume overload

Table I *Clinical features*

AS=aortic valve stenosis AI=aortic valve insufficiency

	Pat 1	Pat 2	Pat 3
Age (y) and sex	54 ♂	35 ♂	21 ♂
Underlying heart disease	AS congenital	AS congenital (bicuspid)	? congenital
Blood culture	α haemolytic streptococci	Str Mutans	Corynebacterium species
Duration of symptoms (weeks)	3	20	3
Antimicrobial therapy	Erythromycin ampicillin streptomycin (54 days)	Penicillin vancomycin (22 days)	Ampicillin gentamycin penicillin (24 days)
Complications	Retinal emboli severe AI	Severe AI	Severe AI
Surgical therapy	Aortic valve replacement	Aortic valve replacement	Aortic valve replacement
Surgical findings	Vegetations on aortic cusps 3×6 mm perforation in left coronary cusp	Vegetations 4 mm in size on destroyed aortic cusps	Vegetations 5 mm in size on aortic cusps perforations in left and right cusp
Outcome	Alive well 26 months after surgery	Alive well 26 months after surgery	Alive well 17 months after surgery

le II *Findings at M mode echocardiography*

ɩ = left ventricular outflow tract

	Pat 1	Pat 2	Pat 3
Vegetations	Shaggy cluster of echoes in aortic root moving into and out of the sound beam could be traced down in LVOT in diastole	Shaggy cluster of echoes in aortic root moving into and out of the sound beam could be traced down in LVOT in diastole	Abnormally strong and thick echoes from aortic cusps could be traced down in LVOT in diastole
Mitral flutter	Present	Present	Absent
Premature mitral closure	Absent	Present	Absent
Septal excursions (mm)	16	12	8
Posterior wall excursions (mm)	10	14	14
Left ventricular end-diastolic diameter (mm)	60	67	56
Fractional shortening of short axis $\frac{(Dd-Ds)}{Dd}$ (0 28–0 41)	0 43	0 38	0 39



Fig 1 M mode scan from the mitral valve (left) to the aortic valve (right) The abnormal cluster of echoes in diastole appears faintly in the left ventricular outflow tract (arrows) and is clearly visible at the cusp level (case 2)

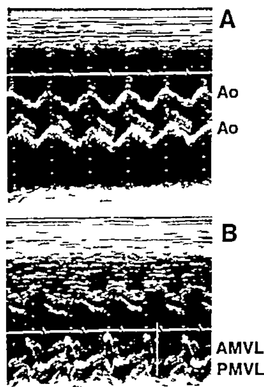


Fig 2 Sections from M mode scans (*A*) The abnormal cluster of echoes at the aortic valve (*B*) Premature closure of the mitral valve *Ao* aorta *AMVL*=anterior mitral valve leaflet *PMVL*=posterior mitral valve leaflet (case 2)

DISCUSSION

Since early diagnosis and treatment of patients with infective endocarditis is essential for successful treatment it is important that single beam echocardiography has proved able to support the diagnosis by demonstrating vegetations on heart valves

The echocardiographic appearance in our patients of an abruptly moving cluster of echoes in the aortic root was similar to that described recently in other reports on aortic valve endocarditis (2 3 5 8 11 16 17 19 20) All three patients presented pathologic echoes in the subaortic left ventricular outflow tract in diastole Patient 2 had a prolapsing aortic valve at surgery as a reasonable explanation for these echoes In the two other patients with non prolapsing aortic valves the abnormal diastolic echoes may be due to lateral resolution distorsion Thus the significance of this sign as to aortic valve prolapse is questionable

Not only aortic valve vegetations but also mitral and tricuspid valve vegetations can be demonstrated by ultrasound (3 17) It is not known how small vegetations can be visualized by echocardiography at present but Dillon et al (3) were able to demonstrate vegetations measuring 3 mm

Patients 1 and 2 showed mitral valve flutter a phenomenon seen in acute as well as in chronic aortic incompetence (18) This sign was not seen in patient 3 A possible explanation is that the regurgitant jet did not strike the mitral valve

Patient 2 presented premature closure of the mitral valve indicating severe acute aortic insufficiency (14) Postoperatively after relief of the volume overload the mitral valve closure was normalized The fractional shortening of the echocardiographic short axis (13) was in all three patients rather high (Table II) in accordance with volume overload to a well functioning ventricle

Thus by single beam echocardiography the diagnosis of aortic valve endocarditis with accompanying aortic insufficiency was reliably established in patients 1 and 2 and strongly suggested in patient 3

At multiple beam cross-sectional imaging the vegetations and their proximal-distal were clearly visualized It was systole and diastole to follow

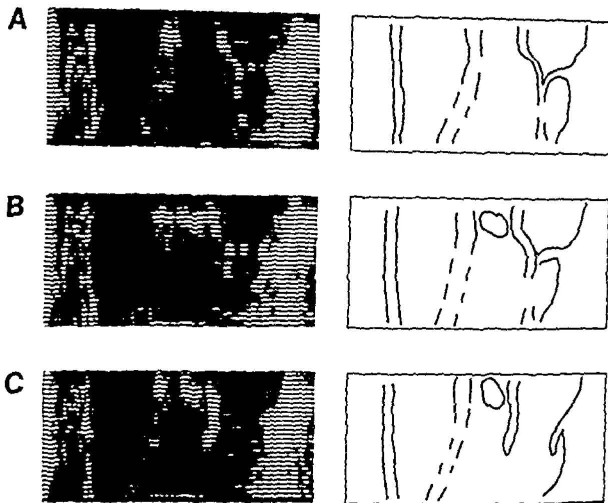


Fig 3 Frames from multiple beam echocardiography longitudinal scanning (*A*) Systole the mitral valve closed no oes in the aortic ring (*B*) 7 frames (140 ms) later the cluster of echoes has descended into the aortic cusp level The mitral valve still closed (*C*) 2 frames (40 ms) later the mitral valve has opened (case 1)

etations perpendicular to the sound beams This is not possible with single beam systems The exaggerated septal excursions were also clearly demonstrated but quantitative assessment of wall movement and demonstration of mitral flutter were not feasible with the present scanner Gilbert et al (6) have likewise demonstrated vegetations and monitored their movements by means of two-dimensional echocardiography achieving a much better picture quality thanks to more advanced equipment

In a report on by far the largest series to date Wann et al (17) suggested that echocardiographically demonstrable vegetations might be a sign of discriminatory value separating patients requiring surgery from those who could be treated conservatively Our small series supports the view that when vegetations are demonstrated the patients are candidates for surgery However with modern high resolution two-dimensional echocardiography this may no longer apply

Using conventional single beam M mode echocardiography Wann et al (17) found vegetations in one third of 62 patients with clinically diagnosed bacterial endocarditis Thus echocardiography apparently is not a sensitive means of diagnosing endocarditis but may demonstrate vegetations of a certain size and a possible complicating aortic insufficiency Since these patients are at high risk of embolism it seems advisable to avoid cardiac catheterization when possible

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Severity of Aortic Stenosis Assessed by Carotid Pulse Recordings and Phonocardiography

Ola Alfred Nesje

From Medical Department B Laboratory of Cardiology University Hospital Rikshospitalet Oslo Norway

ABSTRACT The external carotid pulse the PCG, and the ECG were studied in 26 adult patients with valvular aortic stenosis whose systolic peak pressure gradients ranged from 18 to 165 mmHg A significant correlation was found between the rapidity of the pulse upstroke, as measured by the *T* time, and the location of the peak of the systolic murmur during ventricular ejection, on the one hand, and the gradient, on the other The left ventricular ejection time (LVET) related directly and the pre ejection period (PEP) indirectly with the gradient There was a significant inverse relationship between the PEP/LVET quotient and the pressure gradient but this quotient did not classify the patients according to the severity of the stenosis as well as the *T* time and the location of the peak of murmur When a combination of the *T* time, the PEP/LVET, and the location of the peak of murmur was used in each patient, a good discrimination between the patients was achieved When the pressure gradient was above 50 mmHg at least one of these measurements was abnormal and when it exceeded 100 mmHg at least two measurements were abnormal The study further showed that it is possible to separate patients with valvular aortic stenosis from those with hypertrophic obstructive cardiomyopathy (idiopathic hypertrophic subaortic stenosis) or mitral insufficiency on the basis of carotid pulse tracings and PCGs

Because it changes the externally recorded carotid pulse and phonocardiogram (PCG) in a characteristic way valvular aortic stenosis is well suited for non invasive investigation During ventricular ejection aortic flow increases more gradually and is more turbulent than normal (19) a flow pattern that makes the upstroke of the carotid pulse slow and irregular (5 18) and causes the systolic murmur to peak during middle or late systole (15) Ejection continues until the ventricular pressure has fallen below the level of the aortic pressure and because of the difference between the two pressures in aortic stenosis a delay occurs in the crossing of the two pressure curves and consequently in the closing of the aortic valves (11) The ejection time of the left ventricle becomes longer than normal (3)

In the present investigation the configuration of the carotid pulse the length of the left ventricular ejection time (LVET) and the pre ejection period (PEP) and the timing of the systolic murmur were studied in 26 adult patients with valvular aortic stenosis The principal aim of the study was to find non invasive measurements which might help the clinician in assessing the degree of obstruction in such patients A further aim was to see if any of the measurements might be of help in separating aortic stenosis from some other diseases of the left heart which are accompanied by systolic murmurs The final aim of the study was to find out if it is possible to assess left ventricular function by measuring the systolic time intervals in patients with aortic stenosis

STUDY POPULATION

The patients studied were 8 women and 18 men their ages ranging from 47 to 70 years (mean 60 1) (Table I) All patients were in sinus rhythm Retrograde catheterization of the left heart was performed in all patients and in 22 patients the peak systolic gradient was measured by withdrawing the catheter from the left ventricle to the aorta In 4 patients the left ventricle had to be entered by the transseptal technique and the gradient was assessed

Abbreviations PCG=phonocardiogram ECG=electrocardiogram BP=blood pressure HR=heart rate S D = standard deviation LVET=left ventricular ejection time PEP=pre-ejection period Q A_2=electromechanical systole ΔLVET ΔPEP ΔQ-A_2=deviations from the expected normal values for LVET PEP Q-A_2 ΔpAo= transvalvular aortic pressure gradient A_2=aortic component of the second heart sound max=maximal intensity of the systolic murmur on the PCG

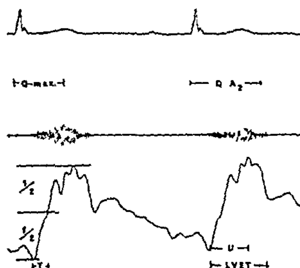


Fig 1 Recording from a patient with valvular aortic stenosis *Top* ECG *Middle* PCG (200 Hz) from the left sternal border *Bottom* carotid pulse registration *Q-max*=distance from the Q wave to the peak of systolic murmur *Q A₂*=electromechanical systole *T*=*T* time *U*=*U* time

by simultaneous measurements in the left ventricle and aorta

Left ventricular angiography was performed in the right anterior oblique position and the ejection fraction was calculated from the cineangiograms by the method of Arbogast et al (1) In two patients (nos 10 and 11) an intracavitary catheter position that permitted the injection of a large bolus of contrast material could not be obtained and the ejection fraction could consequently not be calculated There was an inverse though statistically insignificant relationship between the ejection fraction and the transvalvular pressure gradient ($r=-0.31$ $p>0.05$) Aortography above the aortic valves and selective coronary angiography by the Judkins technique (9) were performed in all patients Patients with mitral disease or more than a small aortic insufficiency i e a slight brush of contrast material in the ventricle during diastole which cleared with the next contraction were excluded Six patients had a 75% luminal stenosis in at least one of the major coronary arteries while the others had coronary arteries without important stenoses

METHODS

A simultaneous registration of the external carotid pulse the PCG at the left sternal border and the ECG was made

Table I *Clinical and catheterization data*

NYHA=classification according to the New York Heart Association LVH=left ventricular hypertrophy according to the ECG ($_SV_1+_RV_5$ or $_RV_5>35$ mV) CM=cardiomegaly (radiological heart volume>500 ml/m² in women 550 ml/m² in men) CAS=coronary artery stenosis EF=angiographic ejection fraction LVEDP=left ventricular end-diastolic pressure Aop=aortic pressure ΔpAo=transvalvular aortic pressure gradient RBBB=right bundle branch block

Pat no	Sex	Age (y)	Exertional An gina	Exertional Dys pnoea	Exertional Syn cope	NYHA	LVH	CM	CAS	EF (%)	LVEDP (mmHg)	Aop (mmHg)	ΔpAo (mmHg)
	♂	58	+	–	+	2	–	–	–	82	8	100/70	18
	♀	68	+	–	–	3	+	–	–	80	5	170/80	20
	♂	70	+	–	–	3	–	–	+	67	25	150/84	38
	♀	56	+	+	–	2	–	–	–	76	12	147/90	40
	♂	57	–	+	–	2	–	–	–	84	5	150/88	45
6	♂	63	+	+	+	3	–	–	–	55	8	126/72	45
7	♀	61	+	+	+	3	+	+	–	71	20	125/75	60
8	♀	52	+	+	–	2	–	–	–	82	12	160/92	60
9	♂	63	–	+	–	2	+	+	+	73	26	108/60	65
10	♂	62	+	+		2	+	–	–		40	180/90	65
11	♂	61	+	–	–	2	+	+	–		14	140/70	70
12	♂	52	+	–	–	2	RBBB	–	–	78	14	156/92	72
13	♂	63	+	+	+	3	+	–	+	57	15	128/79	72
14	♂	62	+	+	–	2	+	+	–	47	13	128/64	77
15	♂	53	–	+	+	3	+	–	–	77	22	140/77	79
16	♀	69	+	+		3	+	+	–	69	17	166/74	80
17	♂	47	+	+		2	+	–	–	87	10	124/78	102
18	♂	58	+	+	+	3	+	–	–	63	16	110/60	102
19	♂	54	–	+	+	3	+	–	–	49	27	110/75	107
20	♀	65	+	+		3	+	–	+	84	12	176/92	105
21	♀	54	–	+		2	+	–	–	75	20	170/80	112
22	♂	63	+	+	+	2	+	–	+	76	20	200/104	120
23	♂	61	+	+	–	2	RBBB	+	+	51	34	98/68	120
24	♂	65	+	+	+	3	+	–	–	54	27	200/106	170
25	♀	65	+	+	+	4	+	–	–	62	13	150/80	135
26	♂	58	+	+	–	3	+	–	–	66	15	160/90	165

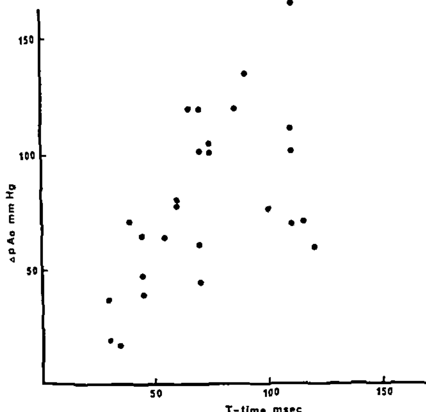


Fig 2 Relationship between uncorrected *T* time and ΔpAo

on a Mingograf 61 direct writing ink recorder The paper speed was 100 mm/sec and the pulse tracings were made with a photoelectrical recorder (Portheine Siemens) which has a time constant of 2 6 sec and a flat frequency response to 400 Hz a frequency response that makes it possible to record high frequency irregularities of the pulse wave (18) The recorder was attached to the bed by a metal bar and all measurements were made on an average of at least 5 heart beats

The pulse recordings were examined for rapidity and irregularity of the upstroke maximal height and contour during middle systole The upstroke rapidity was estimated by the *T* time which is the time needed for the pulse to attain one half of its total height (5) and by the *U* time which is the time required for the pulse to reach its total height (20) (Fig 1) The measurements were corrected for heart rate (HR) by means of Bazett s formula i e the value obtained was divided by the square root of the preceding RR interval in seconds The normal range for the corrected *T* time is 20–46 msec and for the corrected *U* time 50–120 msec (20)

Systolic time intervals were measured according to Weissler et al (21) The total electromechanical systole (Q-A_2) was measured from the onset of the Q wave on the ECG to the first high frequency vibrations of the second heart sound on the PCG LVET was measured from the beginning of the carotid pulse upstroke to the incisura caused by the closing of the aortic valves PEP was obtained by subtracting the LVET from the Q A_2 The expected time intervals according to HR and sex were obtained by using Weissler s formulae (all measurements in msec) Expected Q-A_2 for women 549–2 0×HR standard deviation (S D) 14 for men 546–2 1×HR S D 14 Expected LVET for women 418–1 6×HR S D 10 for men 413–1 7×HR S D 10 Expected PEP for women 133–0 4×HR S D 11 for men 131–0 4×HR S D 13 The deviations of the measured time intervals (ΔQ-A_2 ΔLVET ΔPEP) were obtained by subtracting the measured intervals from the expected intervals The PEP/LVET quotient was measured using uncorrected values of PEP and LVET This quotient is not influenced by HR and sex its normal value is 0 345 with a S D of 0 036 (21)

The distance from the Q wave to the peak of the systolic murmur was measured By subtracting PEP from this measurement the distance from the onset of ejection (A_1) to the peak of murmur was found i e A_1 max=Q-max–PEP The location of the peak of murmur was also expressed as the percentage of ejection time elapsed before the murmur reached its peak i e peaking during LVET=A_1 max/LVET×100 Finally the distance from the end of the murmur to A_2 was measured

The patient series was divided into three groups according to the severity of the stenosis 6 patients with pressure gradients below 50 mmHg 10 patients with gradients between 50 and 100 mmHg and 10 patients with gradients above 100 mmHg Three mea

Table II *Non invasive findings*

Pat no	ΔpAo (mmHg)	RR interval (msec)	*T* time (msec)	*U* time (msec)	Q-A₂ (msec)	ΔQ-A₂ (msec)	LVET (msec)	ΔLVET (msec)	PEP (msec)	ΔPEP (msec)
1	18	1 160	35	120	390	−47	280	−45	110	0
2	20	950	30	220	400	−23	310	−7	90	−11
3	38	730	30	120	325	−49	220	−54	105	
4	40	1 070	45	230	420	−17	325	−3	95	−16
5	45	870	70	210	400	−1	270	−26	130	[illegible]
6	48	770	45	155	385	3	285	5	100	0
7	60	950	70	265	400	−23	305	−12	95	−11
8	60	1 140	120	290	460	17	350	23	110	[illegible]
9	65	1 060	55	160	415	−11	305	−12	110	[illegible]
10	65	690	45	145	365	−2	270	5	105	[illegible]
11	70	930	110	290	470	61	345	53	115	−10
12	72	800	40	220	435	47	335	50	100	−1
13	72	850	115	230	430	33	325	33	105	[illegible]
14	77	1 100	100	300	450	20	350	31	100	−9
15	79	1 030	60	160	400	−24	320	6	80	−25
16	80	780	60	160	360	−35	240	−55	120	15
17	102	1 030	75	240	420	−4	350	36	70	−30
18	102	950	110	240	400	−14	325	19	75	−31
19	102	900	70	220	435	30	325	26	110	6
20	105	700	75	170	360	−17	275	−5	85	−14
21	112	730	110	230	390	5	310	23	80	−20
22	120	1 100	65	215	390	−41	320	1	70	−39
23	120	890	70	260	440	−35	350	51	90	−14
24	120	960	85	215	410	−4	320	14	90	−16
25	135	740	90	240	390	3	310	22	80	−21
26	165	930	110	250	430	11	325	23	95	−10

did not require correction for HR were tested for their ability to distinguish between these groups the uncorrected *T* time the PEP/LVET *quotient and the peaking of* the murmur during LVET The following values were regarded as positive i e indicative of aortic stenosis *T time longer than 50 msec* PEP/LVET smaller than 0 31 peaking of the murmur during the second half of ventricular ejection

RESULTS

Carotid pulse The rapidity of upstroke became slower with increasing obstruction and there was a statistically significant relationship between the *T* time and the pressure gradient (Tables II and III Fig 2) The uncorrected *T* time was longer than 50 msec in all but 2 of the 20 patients with gradients above 50 mmHg The *U* time related less well with the gradient the relationship being significant only for the corrected values

All patients showed high frequency irregularities during the systolic upstroke of the pulse In patients with a small to moderate stenosis and *T* times below 50–60 msec an initial rapid and smooth pulse rise

Table III *Correlation between the non invasive measurements and the transvalvular pressure gradient and the ejection fraction*

	T time	*T* time corr *	*U* time	*U* time corr *	Q-A₂	ΔQ-A₂	LVET	ΔLVET	PEP
ΔpAo (*n*=26)									
r	0.56	0.59	0.35	0.41	0.15	0.29	0.36	0.54	−0.50
p	<0.01	<0.01	n s	<0.05	n s	n s	n s	<0.01	<0.05
Ejection fraction (*n*=24)									
r	−0.24	−0.26	−0.23	−0.22	−0.23	−0.25	0.13	[illegible]	[illegible]
p	n s	n s	n s	n s	n s	n s	n s	n s	n s

* Corrected for HR n s = statistically non significant ($p>0.05$)

PEP/ LVET	Q-max (msec)	A_1 max (msec)	max during LVET (%)
0 39	210	100	36
0 29	220	130	42
0 48	200	85	27
0 29	200	105	32
0 48	300	170	63
0 35	260	160	56
0 31	220	125	41
0 31	280	170	49
0 36	250	140	46
0 39	250	145	54
0 37	330	215	61
0 30	330	230	69
0 37	260	155	48
0 29	320	220	63
0 25	250	170	53
0 50	230	110	46
0 30	300	230	66
0 23	250	175	54
0 34	320	210	65
0 31	270	185	67
0 26	270	190	61
0 22	220	150	47
0 26	310	220	63
0 28	280	190	60
0 26	240	160	52
0 29	280	185	57

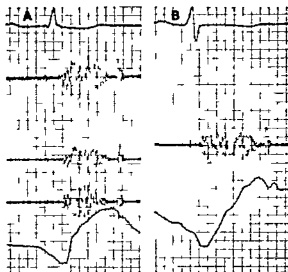


Fig 3 Carotid pulse tracings and PCGs from two patients (*A*) An initial rapid pulse rise is interrupted by a high frequency irregularity which coincides with the onset of the systolic murmur (*B*) A gradual pulse upstroke which is slightly irregular throughout

was interrupted by a sawtooth like irregularity which coincided with the beginning of the systolic murmur (Fig 3*A*) Patients with longer *T* times did not show any rapid pulse rise the upstroke was gradual and slightly irregular throughout (Fig 3*B*) In many of the latter patients however the irregularities increased during the last part of the upstroke (Fig 1) No patient showed a dipping or bifidity of the pulse wave during mid-systole

Systolic time intervals Increasing stenosis was accompanied by a gradual lengthening of LVET and a shortening of PEP (Figs 4 and 5) The relationship between LVET and the pressure gradient was significant only when corrected for HR (ΔLVET) whereas the relationship between PEP and the gradient was significant for both uncorrected and corrected data The presence of coronary artery stenoses did not influence the length of these time intervals

There was an inverse relationship between the PEP/LVET and the gradient (Fig 6) The relation was significant although the scatter was wide Fig 7 explains this scatter It shows that the systolic time intervals deviated in a direction not characteristic for aortic stenosis (abbreviated LVET or prolonged PEP) in several patients with gradients above 50 mmHg and that in two patients both intervals deviated in an uncharacteristic direction resulting in a markedly increased PEP/LVET ratio

The electromechanical systole tended to become longer with increasing gradients but the relationship was far from being of statistical significance

There was no significant relationship between the ejection fraction and the systolic time intervals (Table III) and the relation between the as tolic pressure and the isovolumic ence between diastolic aortic and

ΔPEP	PEP/ LVET	Q-peak	A_1 peak	Peak during LVET
−0 43	−0 52	0 34	0 53	0 52
<0 05	<0 01	n s	<0 01	<0 01
−0 11	0 04	0 27	0 32	−0 17
n s	n s	n s	n s	n s

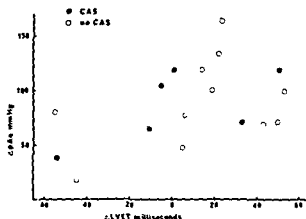


Fig 4 Relationship between ΔLVET and ΔpAo *CAS* = coronary artery stenosis

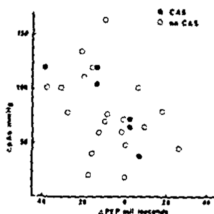


Fig 5 Relationship between ΔPEP and ΔpAo *CAS* = coronary artery stenosis

ventricular pressure) on the one hand and the ejection fraction on the other was even lower

Timing of peak of murmur There was a direct and significant relationship between the location of the peak of murmur during the LVET and the pressure gradient (Fig 8) In 14 of the 20 patients with gradients above 50 mmHg the murmur did not peak until the second half othe LVET The distance from the onset of ventricular ejection (A_1) to the peak of murmur also related significantly with the pressure gradient whereas the distance from the Q wave to the peak did not In all patients the murmur ended at least 10 msec (mean 20 1) before A_2

Combination of measurements The *T* time the

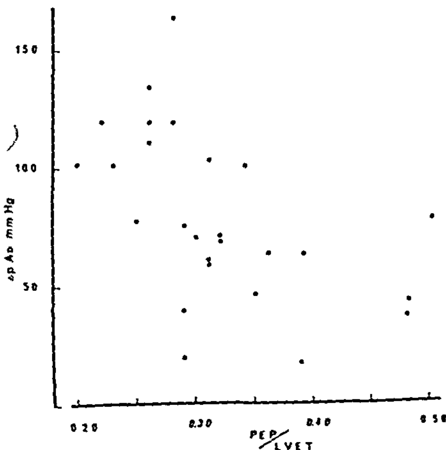
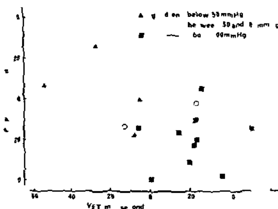


Fig 6 Relationship between PEP LVET ratio and ΔpAo



Fg 7 Dev ations of PEP and LVET from the e p ed normal values accord ng to HR and sex

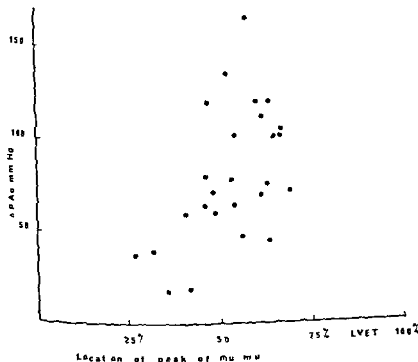
PEP/LVLT and the location of the peak of murmur differed n the r abil t es to dist ngu sh bet een the pat ent groups (Table IV) The *T* t me was most sens t ve and yielded few false negative results The ocation f the peak of murmur discriminated fa rly well between the patients wh le the PEP/LVET gave many false negat ve results especially n the pat ents with grad ents between 50 and 100 mmHg

The best separat on was ach eved by comb n ng the three m a u m r d u ent F g 9) No or n e nent e n 5 of the 6 pa n w hel m Hg t o m a n t e r en itn a gr o t 0 grad e t (6 nt d n b ere n

l

e r t t e f e r 3 eco d a a ly e xe ed t y h *T* d c of the re o h o tr pr s to b a e o h a arten l hyper ten n chan e th f d pu s by n cre s n the se on s m n r relat on to the f rst (6) and t may be d f u t to dec de wh ch max mum to u e for me ng he L me (0)

In the pre ent tud t e LVET wa shortened by as much as 55 msec n one pa ent w th a grad ent of 80 mmHg and was v th n 2 S D from normal in



Fg 8 Relationship between the peak of murmur (of eject on time elapsed before peak ng) and ΔpAo

Table IV *Discriminatory abilities of three non invasive measurements*

ΔpAo (mmHg)	No of pats	Uncorrected T time (msec) <50	Uncorrected T time (msec) >50	PEP/LVET ratio >0 31	PEP/LVET ratio <0 31	Location of maximum of murmur during ejection: First half	Location of maximum of murmur during ejection: Second half
<50	6	5	1	4	2	4	2
50–100	10	2	8	7	3	5	5
>100	10	0	10	2	8	1	9

8 of the other patients with gradients above 50 mmHg These results differ from the findings of other investigators (4 21) and indicate that severe aortic stenosis may be present even if the LVET is normal or shortened In view of the other factors which might influence the LVET this finding was not unexpected While the length of the LVET normally depends on stroke volume (7) it depends on both stroke volume and degree of outflow obstruction in patients with aortic stenosis (2) Variation in the systemic blood pressure (BP) may also disturb the relation between the degree of stenosis and the LVET elevation of the BP by methoxamine has been shown to lengthen the LVET (16) A low stroke volume or a low systemic BP might therefore counteract the lengthening effect of the obstruction on the LVET

The study further shows that patients with severe aortic stenosis have a shorter PEP than patients with a small or moderate stenosis This result agrees with the findings of other investigators (8 21) An adequate explanation for this abbreviation of the PEP in aortic stenosis does not seem to have been found It is improbable that it is due to an increased myocardial contracility as left ventricular dp/dt has been shown to be reduced in patients with aortic stenosis (17) Neither can it be due to a diminished isovolumic pressure as there was no significant correlation between this pressure and the PEP either in the present study or in that by Pansi et al (14)

The fact that the PEP/LVET varied within the normal range (0 31–0 38) in 7 and was increased beyond 0 38 in 2 of the 20 patients with gradients above 50 mmHg shows that a normal or increased PEP/LVET does not preclude important aortic stenosis The diagnostic usefulnes of the quotient depends on the time intervals deviating in the di

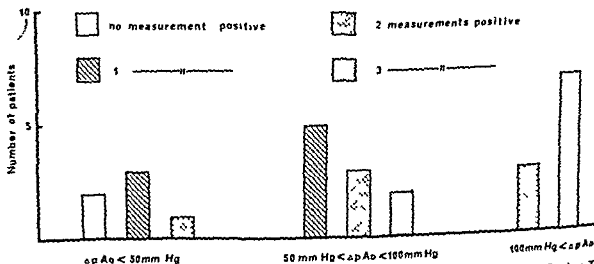


Fig 9 Frequency of positive values of three non invasive measurements in 6 patients with ΔpAo below 50 mmHg in 10 with ΔpAo between 50 and 100 mmHg and in 10 with ΔpAo above 100 mmHg Positive values defined as T time>50 msec PEP/LVET<0 31 and location of peak of murmur during last half of ejection

rection characteristic for aortic stenosis When one or both intervals deviate in the unexpected direction the quotient may lead to false conclusions as to the severity of the stenosis (10)

The present study confirms that a late peaking of the systolic murmur is of diagnostic value for assessing the degree of outflow obstruction in patients with aortic stenosis (4) The location of the peak of murmur during ventricular ejection as well as the distance from the onset of ventricular ejection to the peak had a higher degree of correlation with the pressure gradient than did the distance from the Q wave to the peak of murmur This finding can readily be explained by the influence of the PEP on the Q-peak of murmur distance As the severity of the stenosis relates inversely with the length of the PEP and directly with the distance from the onset of ejection to the peak of murmur these two effects will oppose one another

Several of the present findings suggest that it is possible to differentiate between valvular aortic stenosis and two other left ventricular diseases that are accompanied by a systolic murmur i e hypertrophic obstructive cardiomyopathy (idiopathic hypertrophic subaortic stenosis) and mitral insufficiency by simple non ivasive means In hypertrophic obstructive cardiomyopathy the upstroke is smooth without irregularities and invariably rapid with T times ranging from 20 to 35 msec (13) and a distinct dipping of the pulse wave is often seen during mid systole (13 20) In mitral insufficiency the pulse does not show high frequency irregularities (own observations in 29 patients) the systolic murmur consistently reaches or goes beyond the second heart sound (12) and the systolic time intervals deviate in the opposite direction from aortic stenosis (20)

In cardiac patients without aortic valvular disease the angiographic ejection fraction correlates well with the length of the systolic time intervals a low ejection fraction being accompanied by a prolonged PEP and an abbreviated LVET (21) In the present study however there was no relationship between the ejection fraction and the time intervals although the ejection fraction tended to be lower in the patients with severe stenosis than in those with mild to moderate stenosis This result is probably due to the fact that the outflow obstruction itself and the reduced left ventricular function accompanying it have opposite effects on the length of the systolic time intervals The result suggests that the length of the time intervals is more influenced by the outflow obstruction than by left ventricular function in patients with aortic stenosis and shows that it is not possible to assess left ventricular function by measuring the systolic time intervals in these patients

CONCLUSIONS

The severity of aortic stenosis can be assessed by measurements based on carotid pulse recordings and PCGs As each measurement occasionally yields false negative results it is advisable to use a combination of at least three measurements in the individual patient Valvular aortic stenosis can be separated from hypertrophic obstructive cardiomyopathy or mitral insufficiency on the basis of carotid pulse registrations and PCG

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Electrocardiographic Changes in Right Ventricular Infarction

A Case Report

L R Erhardt and A Sjogren

From the Department of Medicine Serafimerlasarettet Stockholm Sweden

ABSTRACT ST segment elevations in leads CR_4R or V_4R indicating right ventricular (RV) involvement are sometimes seen in patients with acute inferior transmural infarction Whether the RV lesion per se or the concomitant infarction of the posterior septum causes this ECG pattern is unknown We describe a patient with anteroseptal transmural infarction who developed unusually marked ST segment elevations in lead V_4R At autopsy, extensive old fibrotic infarction was found, involving the anterior and lateral RV walls, as well as recent necrosis of the interventric ular septum These findings suggest that the ST segment elevation in V_4R in patients with RV infarc tion may not be caused by the RV necrosis per se but rather by visualization of the posterior septum through the necrotic RV myocardium

We have previously shown that patients with exten sive right ventricular (RV) infarction complicating inferior transmural infarction develop a charac teristic ST segment elevation in the right precordial leads CR_4R or V_4R (1 2) The explanation of this ECG pattern is however not fully understood As the posterior septum is also involved in all of these patients RV infarction (RVI) may merely permit electrocardiographic visualization of the posterior septum through an electric window so that this rather than the RVI per se causes the ST segment elevation in lead V_4R A solution to this question could be obtained by studying ECGs either in pa tients with isolated recent RVI or in those with in ferior left ventricular (LV) infarction and RV in volvement but without any septal involvement Pa tients fulfilling either of these prerequisites are however rare and we have not encountered either pattern in 135 consecutively autopsied patients from our Coronary Care Unit (CCU) using the nitro-BT staining technique Electrical inactivity is however also absent in old RVI and the ECG pat tern and autopsy findings obtained from a patient recently treated in our CCU may therefore throw some light on this problem

CASE REPORT

The patient was a woman aged 68 with a history of two infarcts She was admitted to this hospital because of central chest pain Enzyme patterns confirmed her third acute myocardial infarction (AMI) A representative ECG is shown in Fig 1 revealing atrial fibrillation an incom plete right bundle branch block (RBBB) and ST segment depressions over the LV anterior wall suggesting a sub-endocardial location of the present necrosis The poor R wave progression in the precordial leads was present al ready on admission and thought to be due to her previous AMI

Our patient was readmitted six months later because of chest pain and reinfarction was again confirmed by char acteristic enzyme elevations A representative ECG is shown in Fig 2 revealing a RBBB and in spite of this marked ST segment elevations in the anterior chest leads with a maximum in V_4R and of a degree not usually seen in the absence of inferior LV infarction with RVI The patient subsequently developed complete heart block and received pacemaker treatment but died in progressive heart failure

At autopsy a recent thrombus was found in the anterior descending coronary artery Severe stenoses were noted in the circumflex and right coronary arteries There was massive recent necrosis of the interventricular septum with minor extension into the anterior and inferior LV walls No extension into the right ventricle was noted A transverse heart slice stained with nitro-BT is shown in Fig 3 demonstrating large old infarction of the anterior and lateral parts of the right ventricle Myocardial fibrosis mainly subendocardially was found in the anterior and

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Abbreviations ECG=electrocardiogram RV=right ven tricular RVI=RV infarction LV=left ventricular CCU= Coronary Care Unit AMI–acute myocardial infarction RBBB=right bundle branch block

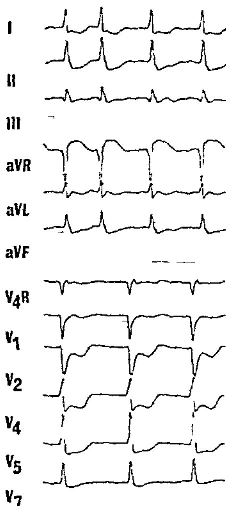


Fig 1 ECG showing atrial fibrillation incomplete RBBB and ST segment depressions in both precordial leads and leads Paper speed 50 mm/s

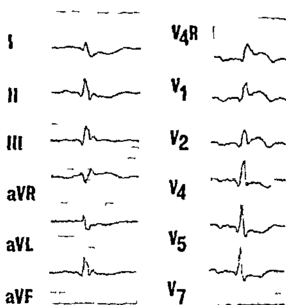


Fig 2 ECG showing ST segment elevations in leads V_4R V_1 and V_2 despite presence of RBBB Paper speed 50 mm/s

inferior LV walls compatible with the previous infarction Microscopic examination of the RV wall verified the gross findings of massive fibrosis mixed with strands of vital muscle cells and no evidence of recent infarction

DISCUSSION

The ECG findings in this patient are compatible with anteroseptal infarction as was confirmed by autopsy However the ST segment rise in lead V_4R was observed to be of considerably higher de



Fig 3 Transverse heart slice stained with nitro-BT showing massive recent necrosis of the interventricular septum and of both papillary muscles in the left ventricle Myocardial fibrosis is present in the left ventricle in both the anterior and the inferior walls An old fibrotic infarction is seen in the right ventricle (arrow) involving the anterior and lateral walls (anterior=upwards)

gree than is usually the case in this type of infarction An ST segment rise in this precordial lead has been found to be a useful sign in the diagnosis of RVI in patients with inferior LV AMI (1 2) In contrast we have not found this pattern useful in the diagnosis of anterior RVI in patients with anterior LV AMI Conceivable reasons for this are 1) lead V_4R may be a poor electrode positioning for the detection of anterior RVIs 2) anterior RVIs rarely attain sufficient proportions to allow ECG diagnosis (1) 3) a moderate ST rise is a common finding in anteroseptal LV AMI irrespective of RVI making this diagnosis difficult or impossible

The unusual ST segment rise in V_4R in our patient may of course merely represent an extreme of the normal pattern seen in anteroseptal infarction However in view of the rarity of such degrees of ST segment rises in this lead in anteroseptal infarcts we prefer to explain the finding as caused by the unusual combination of infarctions found at autopsy i e fresh septal infarction accompanied by old unusually extensive anterolateral RVI This points to the conclusion that ST segment rises in V_4R/CR_4R when associated with RVI are not caused by this lesion but that RVI is necessary for visualization of septal infarction The ST pattern thus remains diagnostic of RVI although not caused by it

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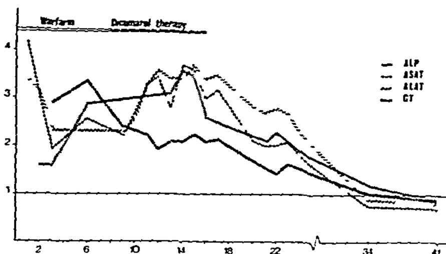


Fig 2 Elevation of liver enzymes in patient 2 (× reference value) in relation to days after ingestion of 50 mg warfarin

COMMENTS

These two patients have three features in common 1) both developed signs of liver cell necrosis and intrahepatic cholestasis which normalized after withdrawal of the anticoagulant drug 2) both have a history of biliary tract disorders and 3) both have a tendency to allergic reactions anaphylaxy to cholegraphy in patient 1 and hay fever in patient 2 Whether their previous hepatic or biliry tract disease is a relevant etiological factor in the present adverse reaction to the drug can only be speculated about Previous reports have mentioned a hepatitis like syndrome induced by the anticoagulants phenindione and phenprocoumon (2 5 7 8 9 10 11) and the mechanism has been regarded as of the cholestatic sensitivity type (12) In our two patients hypersensitivity as well as a possible previous liver damage may have made this effect possible Both patients had a feeling of general malaise which disappeared when the drug was withdrawn Perhaps this favours the suspicion of an immune complex mediated reaction (1)

In conclusion these two case histories suggest that there is also a possibility of a reversible cholestatic syndrome induced by hypersensitivity to warfarin as well as to other anticoagulant drugs

Table I *Serum levels of warfarin in relation to time after ingestion of 50 mg*

Hours after ingestion	Serum level (mg/l)
12	15 0
14	14 7
16	16 8
18	14 7
33	14 2
41	13 2
57	11 8
65	10 2

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Acta Med Scand 204 337 1978

Critical Issues in Modern Medicine—Invited Guest Author's Views—a Suggestion

Can Medicine at its best survive when quantity has taken the place of quality at all levels of education and when there is a steadily growing impact from strong negative forces in society which threaten the patient–doctor relationship? The impact of detailed political and administrative regulation the role of the doctor as a key person in obtaining economic benefits for the patient the dangers of technology running amok the severe threat of financial litigation—all these and many other things should be examined in the coolly detached way of the best research traditions

All these factors are causing increasing concern about the innermost qualities of good medicine

Recently at least in our country and evidently in Britain the increasingly powerful Juggernaut of trade unionism has been gaining an influence on the thoughts action and available time of all those concerned with the care of the sick This may definitely have some positive sides but it has more easily observed negative aspects All this should also be carefully examined

Sir George Pickering s contributions to cardiovascular medicine are well known to most internists in the Scandinavian countries His merits as a constantly alert guardian of the freedom of Medicine seem to me less well known I have on several occasions had the opportunity of hearing George Pickering with bell like clarity expose the threats to good Medicine inherent in all systems of government but particularly in the authoritarian ones An excellent example of civil courage shown by a physician

When inviting Pickering to give a lecture we therefore inquired whether he would like to speak on the subject Patient–physician relationship Deforming influences in society

As seen from the title of his paper Sir George changed and improved our suggestion By his characteristic adroit skill he also brought surgeons into the picture My suggestion Patient–physician relationship would according to British nomenclature of course exclude the surgeons which was by no means my intent However Pickering put doctor in front of patient

As always one may take intense issue with some of Pickering s statements He is certainly provocative

Another thing that keeps bothering me is that Scandinavian Medicine in spite of its numerous excellent outlets for detailed research reports such as the Acta has no suitable international forum for the essential problems facing Medicine Medicine is in part a loyal component of Society in part something which must fiercely defend its own eternal rules This to me is as evident as that Art Literature Philosophy Religion always must stand ready to defend their borders

Such journals as the New England the Lancet the BMJ the Annals of Internal Medicine to mention a few have striven to give us a lot of these things They are all the more essential in times of growing external pressures

Our Chief Editor accepted Pickering s lecture—although it was something out of the ordinary This may have the character of an experiment Whether guest authors should be invited in the future to give their views on important contemporary issues is of course for the editorial board to decide

Bertil Hood Malmo Sweden

Acta Med Scand 204 339–343 1978

THE ESSENCE OF MEDICINE

Doctor–Patient Relationship
The Impact of Recent Changes in Medicine and Society

The Waldenstrom Lecture

I often think that the doctor s objective for his pa tient cannot be better expressed than it was by Thomas Jefferson in 1776 in the Declaration of American Independence— Life Liberty and the Pursuit of Happiness And I go on to think that doctors concentrate too much on the first of these and neglect the others I was moved to this thought by an article by E D Freis (1) which appeared 200 years later in 1976 suggesting that the American *people should restrict their salt intake to 1 gramme* a day in order to stamp out hypertension There is no evidence accessible to me that the measure would achieve its goal But I am sure that it would effectively interfere with liberty and the pursuit of happiness The special foods the special cooking would prevent normal social intercourse and the dreadful monotony of the diet would deprive the sufferer of some of the most easily accessible and least harmful pleasures of life That great physician Franz Volhard had an implicit faith in such a diet and recommended it for nearly everything He cross examined his patients meticulously on their adherence to his instructions Of course the patients insisted on their strict observance of the diet But their fellow patients knew otherwise So arose the expression to be heard among Volhard s patients lying like a saltless

Starting in this way may seem a triviality indeed almost an irrelevance But it expresses my deep conviction that the most important function of the doctor is to make his patients s life happier and more rewarding as well as if possible longer And may I remind you that while the physician has not always the power to prolong life he can always make it happier especially by removing fear and doubt and encouraging hope That is my view of the doctor–patient relationship And whatever changes occur in society and whatever the progress of sci entific knowledge it will remain my view

Much has been written and spoken about the relative importance of the science of medicine and the art of medicine The essence is that knowledge has to be won before it can be used Nevertheless I would like briefly to elaborate Science is a method of acquiring exact knowledge by observation ex periment and measurement leading to the formula tion of a hypothesis to explain the relationship be tween the facts observed Jacques Loeb said in 1924 by a scientific explanation is meant a rationalistic mathematical theory based on quantitative meas *urements Another important property of a sci* entific hypothesis is that it is refutable by experi ment and measurement

All natural laws are laws of probability In the physical world properties can be measured so ex actly that probability is extremely high when a measurement is repeated no matter by whom the results will be as expected Predictions can be made with such confidence that men have been landed on the moon When we come to living matter experi ments and measurements are much less precise Causal factors are difficult to define and more dif ficult to measure a situation that reaches its ex treme expression in the field of animal behaviour Psychiatry is backward not because those who like myself have helped to control scientific policy have failed to realise the importance of psychiatry but because the rewards of scientific enquiry have been so few *The fact is that it is very very difficult*

Art in contrast to science is harder to define precisely and concisely Whereas science is im personal art is intensely personal Like science art is based on experience but here the experience is subjective incapable of precise analysis and impos sible to measure The experience of one individual and its outcome are not necessarily replicable by another What can be done by a Mozart or a Beetho ven a Shelley a Vermeer or a Picasso or by a Yehudi Menuhin cannot necessarily be replicated by others though van Meegeren the forger of Ver meer (that well known connoisseur of art the late Hermann Goering acquired an example) and

Keating the forger of Samuel Palmer (who has lately convulsed the art critics in London) have taught us how imprecise and subjective are all judgements concerning authenticity

The essence of the art of medicine is the relationship between the doctor and the patient It consists essentially in the rapport established between doctor and patient from the first meeting I believe the beginning is all important The doctor should spare no pains to make the patient feel that he is the most important person in the world as indeed at that moment and in that relationship he should be If the patient comes to believe that the doctor is on his side he will do almost anything the doctor asks him to do I agree with Trotter s (3) assessment of the qualities that make a really great doctor The first to be named must always be the power of attention of giving one s whole mind to the patient without the interposition of anything of oneself It sounds simple but only the very greatest doctors ever attain it The second thing to be striven for is intuition This sounds an impossibility for who can control that small quiet monitor? But intuition is only inference from experience stored and not actively recalled The last attitude that I shall mention is that of handling the sick person s mind

Wilfred Trotter was the best doctor I ever knew and worked with He was sometimes described as the surgeon with the mind of a physician His most unusual quality was the way he handled a supposedly difficult patient He always listened with full attention to what the patient had to say Then he made it clear to his patient that he had understood the message After that the difficulties between the patient and her doctors disappeared

My extensive contacts with young doctors in the United Kingdom and the United States have led me to conclude that their greatest single defect is their inability to take a really good history and to be able to sit down and listen to their patients in such a way that they really learn what the patient s problems are that most deeply trouble him For this sad state of affairs several developments in society and in medicine are to blame

Decline in scholarship I have had the privilege of being actively concerned with education since I first became visiting Master in Biology at Westminster School 52 years ago My saddest experience has been the decline of scholarship and the decline in appreciation of the precise use of words The function of language is to convey meaning The modern world of science and technology is flooded with a mounting tide of information that is increasingly precise Surely it must be obvious that if this information is to be used it must be conveyed in language that is as precise and concise as possible But in the contemporary world and particularly in medical education precisely the opposite is happening Examinations increasingly use multiple choice questions that demand answers without evidence The Royal College of Physicians once the repository and bastion of learning now has no test of the candidate s use of language It relies increasingly on M C Q s and other so-called objective tests Detailed scrutiny which I made for my recent book Quest for Excellence in Medical Education revealed that these tests including M C Q s are objective only in so far as the examiners have previously agreed the answers a terrifying return to authority from which Medicine and Science began to escape in Harvey s day 450 years ago

An example of the decline in the precise use of language to convey meaning is the growth of technological jargon In the world inhabited by interns and residents in the United States no one ever walks they ambulate no one ever sweats they diaphorese no one ever coughs up blood they have a haemoptysis

Worse even than jargon is the use of acronyms I remember once an intern presented an old man as suffering from P N D On enquiry I ascertained that the patient did not know what P N D meant and that what he really complained of was attacks of breathlessness at night that wakened him or kept him awake Once I had persuaded the intern to keep his mouth shut we had little difficulty in observing that the patient had Cheyne Stokes respiration I need hardly remind you that this is quite different in causation in prognosis and in treatment from left ventricular failure the diagnosis that the intern tried to convey to me by the term P N D

Why is the bad habit increasing in prevalence? The young do it because they think that long words particularly technical words are scientific and therefore a sign of their learning They add that they are shorter and therefore economical of their time which is untrue

In my deeply considered opinion the decline of language and thus of the ability of patients to communicate with their doctors and of doctors to communicate with their patients and with other doctors is the most serious *disease affecting*

medicine today It is a disease that could be eradicated if teachers and examiners were to make a determined and concerted effort to do so So far there is no sign that they will—more is the pity

The first casualty in doctor–patient relationship is thus the ability to communicate The second is the disappearance of the patient as a person and his replacement by a series of systems or labels This is partly due to the growth of technological jargon but it is also due to the growing importance of the subspecialties and of the laboratory Like so many other changes this has proceeded further and faster in the US than the UK In the US the patient decides that what she needs is a gynaecologist abdominal surgeon cardiologist neurologist or psychiatrist and proceeds to consult one Woe betide her if she is wrong

In the UK the family practitioner still flourishes The patient consults him first If he is competent and a growing proportion are the family doctor may be able to deal with the problem himself If not he should know the specialist best able to help him

As a teacher of medicine I have always maintained that every Professor of Medicine and of Surgery should be reasonably well acquainted with the whole of his subject and an expert on one aspect As T H Huxley said Know something about everything and everything about something As a physician I hold a similar view In my youth I became an expert on peripheral vascular disease I remember with great satisfaction the patients I saved from vascular surgery for example hysteria multiple sclerosis prolapsed intervertebral disc I saved them because I had also been trained in neurology What chiefly terrifies me about medicine in the US is the danger to the patient of falling into the hands of a subspecialist particularly one who uses questionnaires for he starts with a presumed diagnosis and the patient is almost certain to become a disease and cease to be a person *A fortiori* should it happen to be the wrong specialist I am equally terrified by the increasing practice of teaching general medicine by subspecialists No surer method of eliminating the patient as a whole person could be devised

The growth of laboratory procedures has immensely assisted the depersonalisation of the patient The Coulter Counter and the autoanalyser are examples of new machines that have vastly improved the speed precision and range of laboratory procedures Computer Assisted Tomography is the latest and most useful of a long series of advances in radiology that enable the physician to look inside almost as well and infinitely less traumatically than a surgeon has learned to do It is tempting particularly to the recent graduate to perform as many laboratory and X ray examinations as possible and to rely increasingly upon them It is increasingly common particularly in the US for a patient who goes to hospital with a particular complaint to undergo a long expensive and uncomfortable course of diagnoses and treatment for an apparent biochemical aberration and to leave hospital with the original complaint neglected and unaltered—not surprisingly a rather fractious patient

Please do not misunderstand me I am not opposed to specialisations or to the laboratory I merely call attention to the danger that unless their true place is recognised they may extinguish the patient as a whole person

The threats to doctor–patient relationship that I have just considered arise from within the profession I now proceed to two threats which represent the response of the profession to changes in the world at large The first is the response to the threat of litigation The second is the growth of the medical evangelist

At each visit to the US I experience a new wave of revulsion at the extent to which the patient is over investigated and over treated especially by invasive methods that sacrifice his comfort and sense of dignity The reason given is that a failure to carry out these investigations or treatments makes the doctor vulnerable to a charge of negligence for which huge damages may be awarded by the courts As a consequence the doctor is anxious to employ every possible test particularly recently designed ones and to use every treatment for expert witnesses may be called who would testify to the danger to the patient of not doing so So great is the hazard that in California neurosurgeons and orthopaedic surgeons sometimes feel obliged to insure themselves against damages by premiums which may cost as much as $100000 a year Individual surgeons have claims outstanding against them in excess of $1 million

Fortunately the hazard is less pressing and smaller in the UK than in the US The chief reason is the lawyers In the US some law firms interview all patients leaving hospital to see whether they wish to prosecute for negligence If they do and if

lawyers think the case is strong enough the lawyers take the case on the basis that if they lose there is no charge to the patient but if they win they receive a substantial fraction of the damages Fortunately in Britain this practice is disallowed by the legal profession itself

Another response to this threat takes the form of informed consent Medical scientists establish their reputations by their contributions to knowledge These often take the form of experiments on patients Some of those are painful and some are frankly dangerous The worst experiments were those performed by the Nazis on their prisoners especially Jews These revolted all decent folk A convention was held in Nuremberg which laid down a code of practice that should be followed everywhere by those performing experiments on patients One is that the doctor should fully inform the patient of what he proposes to do the benefits likely to accrue from it and the risks involved If the patient then gives his consent the investigation may proceed You would all agree that there can be no harm in this The difficulty arises from what constitutes an experiment It consists in doing something to find an answer that the doctor does not already know Thirty years ago I wrote (2)

If we take a patient afflicted with a malady and we alter his conditions of life either by dieting him or by putting him to bed or by administering to him a drug or by performing on him an operation we are performing an experiment And if we are scientifically minded we should re rd the results Before concluding that the change for r or for worse in the patient is due to the specific tment employed we must ascertain whether the result n be repeated a significant number of times in similar patients whether the result was merely due to the natural history of the disease or in other words to the lapse of time or whether it was due to some other factor which was necessarily associated with the therapeutic measure in question

Strictly speaking a surgeon who performs a graft to by pass a coronary artery occlusion is performing an experiment According to the rules of informed consent he should so inform his patient explaining that it is not really known what the outcome will be because not enough controlled trials of it have yet been made By explaining this he will certainly frighten his patient which according to my code is very bad medical practice The literal interpretation of these rules would in fact provide untold hardship for the sick

Most surgical operations are bad experiments in that there are no controls A random controlled trial is a good experiment in that the controls are chosen at the same time and in the same way as the treated patients the choice between them being a random one Thus a difference in outcome can be attributed to the operation It always offends my intellectual integrity that in the first case informed consent is unnecessary In the second it is necessary I have always taken the view that the best guide to the ethics of a clinical trial or experiment on a patient is would the doctor allow himself or his wife or his daughter to be a subject? If he would it is probably all right If he would not then he is morally wrong to ask his patients

In both these instances the doctor s behaviour to his patient is chiefly guided by the fear of litigation which I regard as an unsound basis for the doctor–patient relationship In every issue that arises the doctor s course of action should depend on what is best for the patient in the immediate and in the remote future I add the remote future because the welfare of today s patients depends upon the successes or failures of yesterday s and it is very important that these successes and failures should be plainly on record

Of all the changes in recent years the greatest threat comes from what one may term the trades union mentality This threat is probably most fully developed in Britain as befits the home of industrial democracy I need hardly remind you that trades unions developed to secure better working conditions and better financial rewards for their members both desirable objects a century ago The weapon that trades unions use is the withdrawal of labour or strike now euphemistally called industrial action The power of the trades unions has been growing rapidly in Britain especially since the accession to power of a Labour government which has passed laws which make the unions virtually masters of society What started as a reform has become an abuse and trades unions tend to dictate to society in their own selfish interests As I write a dispute has paralysed the operating theaters at Dulwich Hospital An orderly persisted in bringing his bicycle into the room where nurses and medical students change to go into the operating theatres He was ordered to remove it by the nurse in charge who was supported by the doctors The union to which the orderly belonged staged a sit in strike demanding the expulsion of the head theatre nurse The dispute continues and surgical operations are

at a standstill As always it is the patient who suffers

This form of behaviour has spread like a virulent epidemic in Britain It has affected doctors and nurses who have it must be admitted been provoked by the kind of episode that I have just described and by a minority Government which is what Labour is and a ruthless Minister determined to introduce her party s programmes into the Health Service despite objections by the doctors and despite her action aggravating the chief limitation on service to the sick namely shortage of money The British National Health Service was a splendid concept which gave an increasingly good service to the sick until 1966 when first the bureaucrats and later the politicians tried to reform it When I worked for the National Health Service namely from its beginning till 1971 decisions were taken in the best interests of the patient Now alas they are taken on political grounds And doctors and nurses have responded by withdrawing or threatening to withdraw their labour the ultimate nadir of the doctor–patient relationship

My last example of a factor disturbing doctor–patient relationship has always been with us It is Fashion Unlike the factors dictating what is a la mode in ladies hats or shoes the changing fashion in medical practice can usually be traced to a man or a group of men who have convinced themselves and are determined to convince others that what is commonly done is wrong and that they are possessed of a new formula guaranteeing salvation I think of them as medical evangelists Sometimes these evangelists have solid evidence behind them Such are those who warn against the evils of cigarette smoking Frequently the evidence is far from complete But that does not deter the medical evengelist Those who believe that cholesterol is poison or who believe that sodium chloride is poison cannot wait till the evidence is complete They have to act and they have to persuade other doctors to do the same

When Sir Thomas Lewis was considering whether to offer me a job he said How do you like Medicine? I said I like it but it disturbs me that I don t understand what I am doing and why It has been a great privilege to be asked by my old friend Professor Hood to give this lecture in honour of an even older friend Professor Waldenstrom I have enjoyed trying to analyse recent changes in doctor–patient relationship and their causation I find it good for my peace of mind to try to understand what we are doing and why

Sir George Pickering D M F R C P F R S
Emeritus Regius Professor of Medicine
Oxford University England

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Natural History and Life Expectancy in Severe Alpha$_1$-Antitrypsin Deficiency, Pi Z

Christer Larsson

From the Department of Internal Medicine Malmo General Hospital University of Lund Malmo Sweden

ABSTRACT Clinical data from 246 adult Swedish individuals with severe α_1 antitrypsin deficiency Pi Z diagnosed in 1963–77, were analyzed Primary emphysema was present in 109 cases Of 75 Pi Z patients with other types of chronic obstructive pulmonary disease (COPD), all but 7 showed signs of emphysema Median age at onset of dyspnoea in Pi Z smokers was 40 years, compared to 53 in non smokers ($p<0.001$) Of the Pi Z individuals over the age of 50 19% had a diagnosis of liver cirrhosis and 15% signs of glomerular renal damage Of 91 deceased patients, 56 died from COPD and 12 from liver disease A greatly reduced survival was demonstrated in Pi Z individuals, regardless of sex Smoking Pi Z individuals had a significantly lower life expectancy than Pi Z non smokers ($p<0.01$)

Key words Alpha$_1$ antitrypsin life expectancy smoking lung diseases obstructive liver diseases

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The association between severe α_1 antitrypsin deficiency and chronic obstructive pulmonary disease (COPD) was recognized 15 years ago (19) An α_1 antitrypsin polymorphism has been well described and there are over 20 known alleles which make up the protease inhibitor (Pi) genetic system (7) Homozygosis of the Z allele (Pi Z) leads to a severe α_1 antitrypsin deficiency and is related to a familial type of panlobular early onset emphysema (5) Evidence has been presented that smoking interacts additively with severe and even intermediate deficiency of α_1 antitrypsin for the development of COPD (2 15 17)

In 1969 Sharp et al (26) recognized the association between the α_1 antitrypsin deficiency state and neonatal liver disease which may progress to juvenile cirrhosis (28) Moreover a considerable number of adult Pi Z individuals develop liver cirrhosis often with malignant transformation (1 6) The majority of Pi Z individuals however reveal no biochemical signs of hepatic dysfunction (16) The occurrence of renal disorders in Pi Z individuals especially children with juvenile cirrhosis has been reported recently (20 21 29) Furthermore some authors have claimed an increased frequency of deficiency phenotypes among patients with rheumatoid arthritis (3) though this finding was not confirmed by a larger Swedish study (27)

No comprehensive clinical follow up study of a large Pi Z patient series has been undertaken previously This report concerns studies of lung liver renal and joint disorders in 246 adult Pi Z patients Special emphasis is placed on the consequences of smoking and survival rate according to the life table method The disease pattern in Pi Z infants has been thoroughly reviewed recently (28) and is not included here

STUDY POPULATION AND METHODS

Blood samples from patients with low serum α_1 antitrypsin levels were drawn in different parts of Sweden and were delivered to the Departments of Clinical Chemistry or Internal Medicine Malmo General Hospital From the period 1963–77 246 cases over the age of 20 years were identified as Pi Z in the Department of Clinical Chemistry where the file of cases was placed at my disposal Serum protein electrophoresis including α_1 antitrypsin measurement was performed according to the methods of Johansson (14) and Laurell (18) Pi Z phenotype was confirmed in all cases by crossed immunoelectrophoresis and starch gel electrophoresis (8) or in recent years by electrofocusing (25)

The 246 Pi Z individuals represented 220 families Twenty six Pi Z relatives were thus included Of these 17 were disclosed in family studies and 9 were accidentally discovered in examinations undertaken for medical reasons All but 4 of the 26 Pi Z relatives eventually

Reprint requests to Dr C Larsson Dept of Internal Medicine Malmo General Hospital S 214 01 Malmö Sweden

Table I *Distribution by sex smoking habits and chronic obstructive pulmonary disease (COPD) among 246 Pi Z individuals*

		Smokers		Non smokers	
		COPD	No COPD	COPD	No COPD
Males	141	84	12	32	13
Females	105	45	10	23	27

suffered from COPD Of the 246 Pi Z individuals 12 were discovered in population screening surveys and 4 of them suffered from COPD The great majority 208 Pi Z patients were discovered in examinations undertaken because of different kinds of manifest medical symptoms (Dr P Mazodier Upplands Väsby contributed 20 patient histories) The distribution by sex and age is presented in Table I and Fig 1

The clinical investigation including physical examination had taken place at the patients local hospitals The case histories were analyzed by the author for information about smoking habits respiratory tract infections cough sputum production dyspnoea as well as for the chronology of these symptoms In addition signs of liver kidney and joint disease were looked for When necessary additional information was obtained by telephone interviews of patients or relatives if the patient was dead Laboratory investigations—including spirometry chest X rays and liver and renal function tests—were performed according to standard procedures and results were available for the majority of patients Special lung function tests were ormed in several cases Bromsulphalein or galactose nce tests and liver biopsies were performed on priate indications Autopsy reports were available 58 of the 91 deceased patients in 40 cases with istological examination

The term COPD denoted the diagnosis of emphysema chronic bronchitis or bronchial asthma The diagnosis of COPD was retrospectively assigned by the author on the basis of available data which in a few cases were incomplete The diagnosis of emphysema was in the great majority of cases based on the existence of permanent dyspnoea on exertion with exclusion of other causes such as heart disease or anaemia Further support for the diagnosis of emphysema was given by physical examination and X ray findings interpreted by the local radiologist Pulmonary function tests were performed in the majority of cases large static lung volumes and signs of irreversible expiratory obstruction were considered consistent with emphysema Primary emphysema denoted the presence of persistent exertional dyspnoea before any history of chronic bronchitis Chronic bronchitis and bronchial asthma were defined according to the WHO definitions (31) The term asthmatic bronchitis was confined to chronic bronchitis with a predominant and reversible bronchospastic component The diagnosis of liver cirrhosis was based on histologic findings or on clear-cut clinical findings The definition of glomerular renal damage was the occurrence of recurrent or permanent proteinuria and/or haematuria where postrenal disorders or infections could be eliminated

Cumulative survival probabilities were estimated using the life table method (4) for the 155 living (84 males and 71 females) and the 91 deceased patients (57 males and 34 females) Twenty years of age was chosen as a starting point for the life tables so all survival probabilities are conditional on this age being reached Since no use was made of the survival information for the Pi Z persons up to the dates of phenotyping the standard life table method had to be modified slightly—an individual entered the calculation at the age of phenotyping The procedure is based upon the number of persons alive at the beginning of a 5-year interval and accounts for the number of those who enter or withdraw alive during the interval This makes it possible to use all survival information accumulated from the date of Pi Z phenotyping up to the closing date of the study Dec 31 1977

Survival curves starting at 20 years of age in the individuals grouped according to sex and smoking habits are presented in Fig 2 Death rates were compared using the log rank test (24) Tekn Dr C J Lamm Department of Mathematical Statistics University of Lund advised on the statistical analyses of survival Information on the main causes of death in all 91 deceased patients (Table II) was available from hospital records autopsy reports or death certificates

RESULTS

Of 184 Pi Z patients with COPD 109 (59%) were classified as having primary emphysema Forty-one of these 109 patients had chronic bronchitis as a later complication 67 (37%) had an initial history of

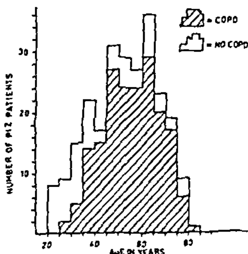
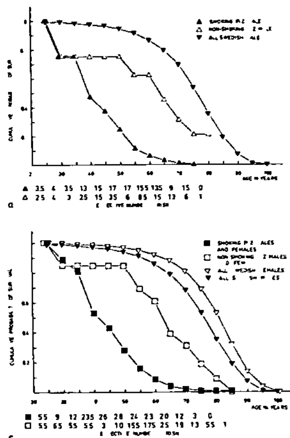
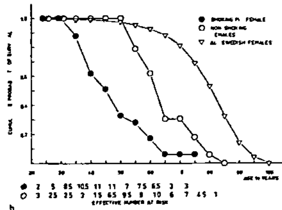


Fig 1 Distribution by age and chronic obstructive pulmonary disease (*COPD*) among 246 Pi Z individuals at the closing date of the study Dec 31 1977 or for the deceased patients at the date of death





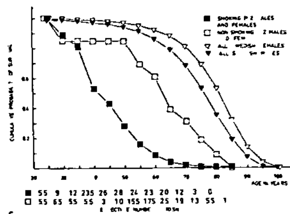


Fig. 2. Cumulative probability of survival given that 20 years of age is reached in smoking and non-smoking Pi Z males (*a*) and females (*b*) and in all smoking and non-smoking Pi Z individuals (*c*). Figures at the bottom imply the effective number of persons exposed to risk of dying during the 5-year intervals.

chronic or asthmatic bronchitis and only 8 (4%) presented with bronchial asthma of extrinsic type. In 68 of the 75 COPD patients without primary emphysema, clinical evidence of emphysema appeared later. Thirty-four patients had an initial history of recurrent pneumonia and 27 of them developed COPD later. Twenty-eight patients, all with COPD, also had a diagnosis of bronchiectasis.

Fig. 3 presents the age at onset of dyspnoea in 169 Pi Z patients with emphysema. Eight patients (all but 2 non-smokers) with a diagnosis of emphysema but no symptoms were excluded. The diagnosis of emphysema was based in 5 cases on autopsy findings and in the 3 patients who were alive on clear-cut radiological and/or physiological findings. There are highly significant differences ($p<0.001$) between smokers and non-smokers (Mann-Whitney's test). The difference in median age at onset of dyspnoea is 13 and 15 years for smoking and non-smoking males and females, respectively.

Twenty-nine (12%) of the 246 Pi Z patients had a diagnosis of liver cirrhosis, in 24 cases biopsy verified. Only one of all the Pi Z patients had knowingly had neonatal hepatitis but no histological signs of cirrhosis were detected at her death at 63 years of age. Twenty-seven of the patients with cirrhosis were over the age of 50 at the time of the diagnosis, constituting 19% of all Pi Z individuals in that age group. Only 3 of them had a history of heavy alcohol consumption. Eight patients, all with cirrhosis, also had malignant hepatoma according to autopsy reports.

Thirty-seven (15%) of the 246 Pi Z patients showed signs of glomerular renal damage, manifested by constant or recurrent proteinuria or haematuria. Three patients eventually suffered from advanced renal failure, which was the direct cause of death in one. Microscopic post-mortem examination performed in 2 of them showed advanced proliferative glomerulonephritis. In the great majority of the 37 cases, however, the serum creatinine level was normal.

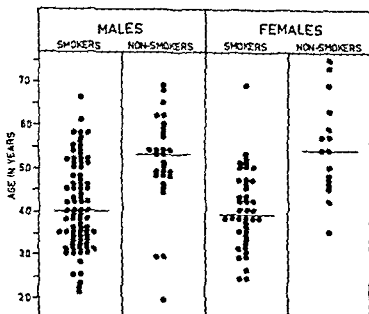


Fig 3 Age at onset of dyspnoea in 169 Pi Z patients with the diagnosis of emphysema Horizontal lines indicate median values

Eleven Pi Z patients (9 females and 2 males) suffered from rheumatoid arthritis rheumatoid factor being present in 7 Another 7 had a history of considerable joint pain but no confirmed diagnosis One female had ankylosing spondylitis and 2 other females had systemic lupus erythematosus

As shown in Fig 2 the survival rate was higher or Pi Z non smokers than smokers regardless of in all age groups above 35 According to the log test the death rate was significantly higher for ing than for non smoking Pi Z males ($p<0.01$) e difference in death rates between smoking and non smoking Pi Z females (Fig 2*b*) approached significance ($p=0.06$) before the age of 50 but no difference was found thereafter All smoking Pi Z individuals (Fig 2*c*) had a significantly lower survival rate than Pi Z non smokers ($p<0.01$) Compared to the normal Swedish population smoking and non smoking Pi Z females and smoking Pi Z males had highly significant survival disadvantages ($p<10^{-7}$) The statistical disadvantage for non smoking Pi Z males was not of the same magnitude ($p<0.01$)

The main causes of death in the 91 deceased Pi Z patients are given in Table II Fifty six patients died of COPD and 12 of liver disease All 4 patients with terminal pulmonary embolism suffered from advanced COPD Nine deceased patients (10%) had no diagnosis of COPD all but one of them were over the age of 50 and had never smoked Autopsy reports were available for 5 of them Of the patients dying from COPD 45 were smokers and only 11 non smokers Eighteen of the patients dying from other reasons were smokers and 17 non smokers Hence non respiratory causes of death were more common among non smokers than smokers

Table II *Causes of death in 91 patients with α₁-antitrypsin deficiency Pi Z*

Cause of death	No of pats
Chronic obstructive pulmonary disease	
Respiratory insufficiency	54
Pneumothorax	2
Liver cirrhosis with or without primary hepatoma	
Hepatic failure	9
Bleeding oesophageal varices	3
Miscellaneous	
Pulmonary embolism	4
Bronchopneumonia	3
Renal failure	1
Myocardial infarction	1
Congestive heart failure	2
Subarachnoidal bleeding	2
Intracerebral vascular lesion	2
Subdural haematoma	2
Malignant melanoma	1
Status epilepticus	1
Peritonitis	2
Drowning	1
Suicide	1

DISCUSSION

Since Laurell and Eriksson first described the association between COPD and inherited α_1 antitrypsin deficiency in the early 60s (19) and Eriksson defined the familial panlobular early onset emphysema (5) a great many reports have confirmed this association After 1969 when Sharp et al (26) recognized a neonatal hepatitis syndrome in Pi Z children a considerable number of elderly Pi Z patients with liver disease have also been reported (1 6) Several other organ manifestations have been described in the deficiency state (3 10 11 20 21 23 27 29 30)

The aim of the present study—to give the natural history of the Pi Z deficiency state—was hampered to some extent by the unavoidable element of selection of the 246 Pi Z individuals 217 were hospital patients the other 29 cases were disclosed in family or population surveys but only 10 of them were free from disease at the end of 1977 Assuming a Pi Z frequency of 1/1 500 (5 28) the Swedish population should contain 4 000 Pi Z individuals over the age of 20 The problem of selection can be avoided only in prospective studies requiring very lengthy follow ups An extensive Swedish study of Pi Z newborns has been initiated recently (28)

The selection mechanism affects the comparison of life expectancy in Pi Z and all Swedish individuals (Fig 2) The statistical disadvantages in death rate are highly significant ($p<10^{-7}$) for smoking Pi Z males and all Pi Z females as compared to the normal population These differences pertain to the Pi Z population investigated but not necessarily to the total Pi Z population The selection mechanism may affect the comparison between smoking and non smoking Pi Z individuals too but in this case no serious bias is likely to be produced If one excludes the 10 Pi Z persons disclosed in family and population surveys who were free from symptoms at the end of 1977 the significant differences in survival still persist

A serious kind of bias is avoided by disregarding survival information up to the age of phenotyping Only information obtained by observing an individual from that point of time onwards is recovered in the life table calculations The problem of selection recurs when calculating the prevalences of different disorders in α_1 antitrypsin deficient individuals Healthy Pi Z persons are certainly underrepresented in the present series and to an unknown extent A large number of Pi Z patients who sought medical care primarily for symptoms unrelated to pulmonary disease developed COPD later Under the age of 40 only 21 of 54 persons (39%) had developed COPD (Fig 1) whereas over that age 163 of 192 individuals (85%) suffered from COPD a fact which strongly supports the idea that most Pi Z individuals eventually develop COPD The low prevalence of COPD in the non smoking females (Table I) is remarkable Statistical studies have not been performed due to lack of matched age groups Even elderly non smoking females seldom develop pulmonary symptoms Of the 29 patients with liver cirrhosis all but 7 (6 non smokers) had COPD

Earlier reports of liver disease in adult Pi Z patients have suggested the prevalence of liver cirrhosis to be about 10% (6) In the present study about the same proportion 12% of all adults had cirrhosis and a further finding of interest is the high prevalence 19% in Pi Z patients over the age of 50 In addition a considerable number of Pi Z patients in this survey revealed clinical signs of unspecific hepatopathy or advanced steatosis/fibrosis in biopsy specimens Thus ultimately the risk of developing liver cirrhosis is substantial for adult Pi Z persons and increases with age The pathogenetic mechanism is unknown (16) Quite remarkable is the absence of any history of neonatal hepatitis in any of the 29 cirrhosis patients in this study The only Pi Z female who had knowingly had neonatal hepatitis syndrome died 63 years old slight fibrosis without signs of nodular regeneration was found at autopsy

The high incidence of glomerular renal damage in the present Pi Z population (15%) can hardly be explained by a co-existent hepatic disease since only 5 of the 37 cases with glomerulopathy had a diagnosis of liver cirrhosis No signs of glomerular affection were found in the remaining 24 patients with cirrhosis In contrast to 3 Pi Z patients suffering from advanced renal failure the remaining 34 patients with renal affection had no signs of grossly impaired renal function It should be noted however that exhaustive tests of renal function have been performed hitherto in only a minority of the affected patients No conclusions can be drawn at present about the natural history or prognosis of the glomerular affection in α_1 antitrypsin deficiency It seems however usually not to be a serious clinical problem but should be further investigated

The influence of Pi polymorphism on the inci

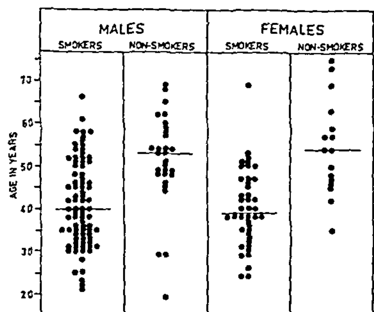


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Subdural haematoma	2
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Drowning	1
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Intermediate Alpha$_1$-Antitrypsin Deficiency, Pi M−

Christer Larsson

From the Departments of Internal Medicine and Clinical Physiology Malmo General Hospital University of Lund Malmo Sweden

ABSTRACT A 50-year-old man was found to have 40% of the normal serum α_1 antitrypsin concentration but subsequent electrofocusing showed a pattern indistinguishable from the ordinary M pattern This finding and family studies suggested that he was a carrier of a null (−) allele Heavy smoking and this Pi M− phenotype in interaction probably were responsible for the development of emphysema documented by an extensive investigation of lung function Two non smoking offspring carrying the null allele had normal lung function Normal karyotypes were found in all the Pi M− subjects

Key words Alpha$_1$ antitrypsin pulmonary emphysema

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A large number of co-dominant alleles determine the structure and concentration of serum α_1 antitrypsin So far more than 20 genetic variants or protease inhibitor (Pi) types have been identified (4) It has long been known that severe α_1 antitrypsin deficiency Pi Z is associated with early onset of pulmonary emphysema (3) Recently intermediate deficiency Pi MZ was suggested to interact with smoking in the development of emphysema (11) In 1973 a carrier of Pi− (null null) phenotype with no detectable α_1 antitrypsin in serum and with advanced emphysema was first reported (19) Since then several other reports of an apparent null gene in the heterozygous state (Pi M−) have appeared (1 13 16) Lieberman et al (15) reported recently early emphysema in a Pi M− subject

Since the Pi− (null) allele corresponds to an almost zero concentration of α_1 antitrypsin the heterozygous phenotype Pi M− cannot be established at present by blood sample phenotyping alone It can only be suspected from family studies and the finding of approximately half the expected serum concentration of α_1 antitrypsin

This report concerns a family study on a 50-year-old man with Pi M− phenotype discovered at a preventive medical unit during screening for intermediate α_1 antitrypsin deficiency Lung function studies revealed early emphysema Since there have been several reports of an association between α_1 antitrypsin variants and sex chromosome mosaicism (6 8) chromosome analysis was performed on the Pi M− proband and his 3 offspring all with M− phenotype

MATERIAL AND METHODS

Serum protein electrophoresis including α_1 antitrypsin measurement was performed at the Department of Clinical Chemistry according to the methods of Johansson (7) and Laurell (12) on fresh blood samples Pi typing was performed according to the method of Pierce et al (17) using electrofocusing Chromosome analysis was performed by Dr F Mitelman Department of Clinical Genetics University Hospital Lund by means of the Giemsa trypsin banding technique (18) Ordinary chest X rays and laminograms were available and were examined by Dr C Hellekant Department of Radiology Lung function was studied by previously reported methods (10 11) including ^{133}Xe radiospirometry

CASE REPORT

The proband was discovered in a population study in which all 50-year-old men living in the city of Malmö Sweden were invited to the Division of Preventive

Reprint requests to Dr C Larsson Dept of Internal Medicine Malmo General Hospital S 21401 Malmo Sweden

Abbreviations VC=vital capacity TLC=total lung capacity FRC=functional residual capacity RV=residual volume MVV$_f$=maximal voluntary ventilation FEV$_1$=Forced expiratory volume in 1 sec FIV$_1$=forced inspiratory volume in 1 sec WOV=wash-out volume LCI=lung clearance index CV=closing volume CC=closing capacity PMI=pressure maximal inspiration CR=coefficient of retraction C_{dyn}=dynamic compliance Pa O_2=arterial oxygen tension Pa CO_2=arterial carbon dioxide tension ΔAa PO_2=alveolo-arterial O_2 difference V_D/V_T=dead space/tidal volume DL_{CO}=diffusing capacity for CO

Table I *Serum α_1-antitrypsin concentration and Pi types in family members*

I_1=proband I_2=his wife II_1-II_3=offspring

Family member	Sex	Height (cm)	Age (y)	Serum α_1 antitrypsin (% of normal)	Pi type
I_1	♂	184	50	40	M–
I_2	♀	160	47	140*	MM
II_1	♂	199	26	50	M–
II_2	♂	199	21	70	M–
II_3	♀	185	18	60	M–

* Alcoholic liver cirrhosis

Medicine General Hospital for a screening procedure that included serum protein electrophoresis (11)

The proband is a heavy cigarette smoker with a history of 28 pack years consumption He had no history of chronic bronchitis but reported dyspnoea on climbing stairs or slopes in the last 5 years He had been admitted to hospital in 1974 with a diagnosis of Bell's facial paralysis A chest X ray at that time showed hyperinflation of the lungs Physical examination in 1976 revealed a 184 cm tall man with normal chest form Auscultation of the lungs revealed normal findings and no rales or ronchi could be heard

His three children were very tall and thin but had no skeletal deformities They were all non smokers and had no clinical signs or symptoms of pulmonary disease The mother of the proband was deceased and his father was unknown He had no contact with his half siblings

RESULTS

rum α_1 antitrypsin concentrations and proposed Pi types of the proband and his family members are given in Table I and Fig 1 The Pi M– individuals of the family had electrofocusing patterns indistinguishable from the ordinary Pi M phenotype pattern The assumed Pi– allele thus did not influence the electrofocusing pattern Chromosome analyses showed completely normal karyotypes in all family members

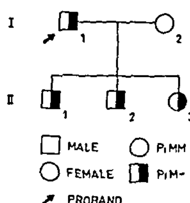


Fig 1 Pedigree of the family

An extensive evaluation of lung function in the proband is summarized in Tables II and III The examination revealed greatly increased static lung volumes (VC TLC FRC and RV) with an increased RV/TLC% and moderate expiratory obstruction as judged from a low FEV_1/VC% A loss of lung elastic recoil was indicated by the abnormal PMI, CR and C_{dyn} values The findings were consistent with emphysema with secondary airway obstruction Signs of abnormal intrapulmonary gas distribution and gas exchange (WOV LCI

Table II *Spirometric findings in the proband*

	Measured value	% of predicted normal value	Deviation from predicted value (S D)
VC (l)	6 6	122	+2 1
TLC (l)	10 5	134	+3 6
FRC (l)	5 9	150	+3 0
RV (l)	3 9	169	+3 0
FRC/TLC (%)	56	113	+1 1
RV/TLC (%)	37	122	+1 2
MVV_F (l/min)	148	85	−0 9
FEV_1 (l)	3 6	86	−1 2
FEV_1/VC (%)	55	71	−3 3
FIV_1 (l)	5 7	116	+1 4
FIV_1/VC (%)	86	96	−0 7
WOV (l)	68	210	+6 0
LCI	11 6	147	+3 0

Table III *Closing parameters lung mechanics and gas exchange at rest in the proband*

	Measured value	Predicted value ±2 S D
CV/VC (%)	28 6	14 7±7 4
CC/TLC (%)	44 3	41 2±8 2
Slope of phase III (ΔN_2%/l)	3 0	1 6±1 4
PMI (cmH_2O)	19 8	21 9±9 6
CR (cm H_2O/l)	1 89	3 15±1 45
C_{dyn} (l/cm H_2O)	0 308	0 704±0 162
Pa O_2 (kPa)	10 0	11 0±1 9
Pa CO_2 (kPa)	4 9	5 0±1 0
ΔAa PO_2 (mmHg)	37 7	14 0±12 2
V_D/V_T	0 44	0 30 0 15
DL_{CO} (ml/min/mmHg)	9 7	13–22 (normal range)

ΔAaPO₂ V_D/V_T DL_{CO}) were present ^{133}Xe radiospirometry showed ventilation abnormalities which could suggest the existence of basal emphysema most pronounced on the left side A chest X ray with laminogram showed marked hyperinflation but no significant decrease in vascular markings

In two of the offspring (II_1 II) a complete examination of lung function showed normal results The lung function of the third offspring was not examined

DISCUSSION

The present investigation of serum α_1 antitrypsin concentrations and phenotypes in the family members indicates that all the three offspring had inherited the antitrypsin deficiency from the proband (Fig 1 Table I) We assumed their phenotypes to be Pi M− After several investigators (1 13 16 19) had shown the existence of a null gene others (2 9 14) have reported α_1 antitrypsin variants with very low serum levels and with almost the same mobilities as the ordinary M protein In liver biopsy specimens periodic acid Schiff positive inclusions have been shown to be produced by at least one of the abnormal M variants M_{Duarte} (14) in contrast to the null allele (5)

The possibility of existing gross chromosomal abnormalities has been ruled out by the finding of normal karyotypes in the Pi M− members of this family This finding however by no means excludes the possibility of a chromosomal deletion of the allele that ordinarily determines the production of α_1 antitrypsin A single deletion cannot be detected even with modern banding techniques

It is obvious from this and other reports that heterozygotes for the null allele cannot be detected by blood sample phenotyping alone (starch gel electrophoresis with subsequent crossed immunoelectrophoresis or electrofocusing) A quantitative determination is necessary The Pi M− phenotype should be suspected from family studies and the finding of approximately half the expected serum concentration of α_1 antitrypsin There should be no other allele product visible on electrofocusing

The fact that the Pi M− proband in this study had physiological evidence of pulmonary emphysema suggests that even heterozygotes for the unusual null allele run an increased risk of developing emphysema The contribution of the proband's heavy smoking history was probably substantial No signs of emphysema were detected in two offspring with the same phenotype However their low ages (21 and 26 years) and the fact that they were both non smokers must be taken into account

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The Preferential Role of Triiodothyronine in the Regulation of Basal Metabolic Rate in Hyper- and Hypothyroidism

Klaus Johansen Jens Mølholm Hansen and Lis Skovsted

From the Department of Internal Medicine and Endocrinology Herlev Hospital Herlev Denmark

ABSTRACT The free triiodothyronine index (FT_3I) was significantly correlated to basal metabolic rate (BMR) in hyperthyroid ($r=+0\ 63$, $p<0\ 01$) and hypothyroid patients ($r=+0\ 61$, $p<0\ 05$) Elimination of the effect of the free thyroxine index (FT_4I) on the total correlation between BMR and FT_3I by partial correlation analysis gave partial $r=+0\ 60$, $p<0\ 01$ in hyperthyroid patients and partial $r=+0\ 43$, $p<0\ 1$ in hypothyroid patients The FT_4I did not correlate to BMR in either hyper or hypothyroid patients These results point to triiodothyronine as the major regulator of BMR in hyper and hypothyroidism

Key words basal metabolic rate hyperthyroidism hypothyroidism thyroxine triiodothyronine

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The intriguing but speculative concept that thyroxine (T_4) exerts physiologic activity by acting solely as a prohormone to provide triiodothyronine (T_3) was initiated by Gross and Pitt Rivers (6) Renewed interest in the problem was aroused by the demonstration by Braverman et al (2) that in man T_4 to T_3 conversion does in fact occur in the periphery and by the fact that T_3 is biologically more potent than T_4 (14) It is still unclear however whether T_3 is the sole active thyroid hormone or whether T_4 also plays a physiological role as an active thyroid hormone Attainment of an euthyroid state by elevated or normal T_3 concentrations in the presence of reduced T_4 concentration in the blood has repeatedly been shown in patients with endemic goiter (3 18 23) Hashimoto s disease (5) and Graves disease after treatment with propylthiouracil (10) or radioactive iodine (22) These data show at least that T_3 is able to maintain a euthyroid state despite subnormal serum T_4 levels Moreover the occurrence of hyperthyroidism associated with raised serum T_3 and normal serum T_4 concentrations has been well documented (8) Studies on the relation ship between thyroid hormone levels in blood and their peripheral effects are sparse (11)

In order to get further insight into the relative roles of serum T_4 and T_3 in the regulation of basal metabolic rate (BMR) we studied the degree of correlation between serum T_4 and T_3 and BMR in patients with hyper and hypothyroidism The concept was that the major regulator of BMR would exhibit the highest degree of correlation with BMR and vice versa

PATIENTS AND METHODS

BMR serum T_4 and serum T_3 and T_3 resin uptake were measured in 19 newly diagnosed untreated patients with hyperthyroidism (7 males 12 females) and in 14 patients with newly diagnosed untreated primary hypothyroidism (2 males 12 females) The patients were randomly selected from those referred to our department during 1 year The diagnosis of hypo- or hyperthyroidism was established by the presence of typical clinical manifestations favorable response to therapy and was supported by appropriate laboratory abnormalities based on the reference value (mean±2 S D) of our laboratory In the hypothyroid subjects 3 of the following criteria were fulfilled serum free T_4 index (FT_4I) ≤4 4 arbitrary units serum free T_3 index (FT_3I) ≤130 arbitrary units BMR ≤90% and serum thyrotropin (TSH) ≥5 μU/ml In the hyperthyroid patients 2 of the following criteria were fulfilled serum FT_4I >11 arbitrary units serum FT_3I >210 arbitrary units and BMR >115%

Reprint requests to K Johansen Department of Internal Medicine and Endocrinology Herlev Hospital DK 2730 Herlev Denmark

Abbreviations T_3=triiodothyronine T_4=thyroxine FT_3I=free T_3 index FT_4I=free T_4 index BMR=basal metabolic rate TSH=thyrotropin

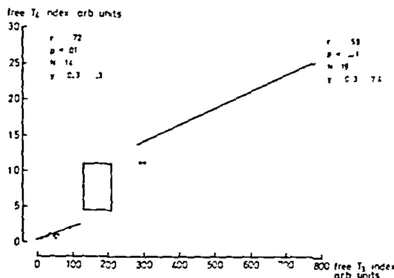


Fig 1 Correlation between FT_4I and FT_3I in untreated patients with hypo- and hyperthyroidism Hatched area = normal range

FT_4I and FT_3I were evaluated using the product of the total hormone concentrations and the T_3 resin uptake (7) Serum T_4 was determined by a modification of the Murphy method (20) serum T_3 (21) and TSH (24) by radioimmunoassay BMR was measured by conventional technique with determination of oxygen consumption in the resting patient after an overnight fast and expressed as a percentage of a standard value for persons of the same age sex and surface area using the Harris Benedict standards (4)

The coefficients of variation (%) of the laboratory determinations were the following in the hyper and hypothyroid patients respectively serum T_4 5 and 4 serum T_3 5 and 8 T_3 resin uptake 3 and 6 BMR 3 and 4 All T_4 and T_3 values in serum were determined in one assay Partial regression analysis was performed according to conventional statistical procedures (1)

RESULTS

19 hyperthyroid patients had a mean BMR of 132±11% (S D) mean FT_4I of 18±6 and mean FT_3I of 457±142 arbitrary units The corresponding figures in the 14 hypothyroid patients were 80±7 1 6±1 0 and 57±29

The FT_4I and the FT_3I were significantly correlated in both hypo- ($r = +0\ 72$ $p<0\ 01$) and hyperthyroid patients ($r = +0\ 59$ $p<0\ 01$) (Fig 1) and the slopes of the regression lines were identical The regression lines were parallelly displaced and cut the Y axis at a FT_4I of 0 3 in the hypothyroid and 7 4 in the hyperthyroid patients

The results of the correlation analyses are summarized in Table I No significant correlation was found between BMR and FT_4I in the hyperthyroid patients and the correlation dropped further when the effect of FT_3I was eliminated by partial regression analysis The correlation between BMR and FT_3I is significant also when the effect of FT_4I was eliminated

Similar results were obtained in the hypothyroid patients No correlation was obtained between BMR and FT_4I After elimination of the effect of FT_3I the regression coefficient dropped further However the correlation between BMR and FT_3I was significant but when the FT_4I effect was eliminated only at the 10% level

DISCUSSION

The finding of a positive correlation between FT_4I and FT_3I in both hypo- and hyperthyroid patients

Table I *Total and partial regression analysis*

	Hyperthyroid pats (N = 19)		Hypothyroid pats (N = 14)	
	Total	Partial	Total	Partial
FT_4I vs BMR	$r = +0\ 28$ p = n s	$r = -0\ 14$ p = n s	$r = +0\ 43$ p = n s	$r = -0\ 02$ p = n s
FT_3I vs BMR	$r = +0\ 63$ $p<0\ 01$	$r = +0\ 60$ $p<0\ 01$	$r = +0\ 61$ $p<0\ 01$	$r = +0\ 48$ $p<0\ 1$

(Fig 1) is in accordance with several investigations (9 15 17 19)

As FT_4I and FT_3I are interdependent variables that correlate with each other their respective correlations with BMR might simply reflect their interdependence Consequently it is necessary to perform partial correlation analyses which take into account the interdependence of FT_3I and FT_4I and eliminate one of the two parameters when the other is correlated to BMR

The importance of measuring serum T_3 concentrations when hyperthyroidism is suspected is well established Raised levels have been found in hyperthyroidism with remarkably little overlap with values in euthyroid patients (12 16) The association of normal serum T_3 and low serum T_4 values in so-called prehypothyroid euthyroid subjects with mildly elevated serum TSH concentration (13) and in yet euthyroid patients overtreated with antithyroid drugs (10) or radioiodine (22) suggests that serum T_4 levels are more satisfactory for the early diagnosis of these conditions

Our findings of a positive total correlation between FT_3I and BMR also when the effect of FT_4I is eliminated from the total correlation in hyperthyroid patients (Table I) together with the fact that FT_4I does not correlate to BMR seem to indicate that serum T_3 is the major regulator of BMR and a more specific criterion for the diagnoses of manifest hypo- and hyperthyroidism

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Myxoedema and Thyrotoxicosis Relations between Clinical State and Concentrations of Thyroxine and Triiodothyronine in Blood

Klaus Johansen Jens Mølholm Hansen and Lis Skovsted

From the Department of Internal Medicine and Endocrinology Herlev Hospital Herlev Denmark

ABSTRACT The clinical manifestations in thyrotoxic and myxoedematous subjects were assessed by clinical diagnostic score indices and related to the free thyroxine index (FT_4I) and the free triiodothyronine index (FT_3I), basal metabolic rate (BMR) and in the hypothyroid patients to serum thyrotropin (TSH) level The clinical score index was significantly correlated to both FT_4I and FT_3I in both groups of patients No difference existed in degree of correlation between the clinical score index on the one hand and FT_3I and FT_4I, on the other, in either thyrotoxic or myxoedematous subjects The degree of correlation between clinical score index and FT_3I and FT_4I was higher than that between the thyroid hormones and BMR The clinical score index thus appears to be a better indicator of severity of hyper and hypothyroidism than BMR Serum TSH concentration was not correlated to the clinical state

Key words basal metabolic rate clinical score myxoedema thyrotoxicosis thyroxine thyrotropin triiodothyronine

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Raised levels of serum triiodothyronine (T_3) have been found in hyperthyroidism with remarkably little overlap with values in euthyroid subjects (10 11) and hyperthyroidism associated with raised serum T_3 and normal serum thyroxine (T_4) concentrations has been well documented (5) This emphasizes the importance of measuring serum T_3 when hyperthyroidism is suspected The association of normal serum T_3 and low serum T_4 values in so-called prehypothyroid euthyroid subjects with mildly elevated serum thyrotropin (TSH) concentrations (8 9 16) suggests that serum T_4 measurements are more satisfactory for the early diagnosis of myxoedema

In a previous paper (6) we showed that both in overt thyrotoxicosis and in overt myxoedema the free concentration of T_3 in serum expressed as the free T_3 index (FT_3I) is significantly correlated to the basal metabolic rate (BMR) while the free T_4 index (FT_4I) is not T_3 therefore seems to be of major importance in the control of BMR

It might be assumed that the clinical state in overt thyrotoxicosis and myxoedema would also be more closely related to the level of T_3 than to that of T_4 in blood The relative roles of T_3 and T_4 in the control of the clinical features of untreated hyper- and hypothyroidism are unknown Knowledge of this relationship would add to the understanding of the peripheral function of the thyroid hormones

The aim of the present study was to examine the relation of the clinical manifestations to both FT_3I and FT_4I

PATIENTS AND METHODS

BMR serum T_4 T_3 TSH and T_3 resin uptake were measured in 13 newly diagnosed untreated patients with primary myxoedema (11 females 2 males aged 22–87 years) and in 28 patients with newly diagnosed untreated hyperthyroidism (20 females 8 males aged 19–76 years) The patients were randomly selected from those referred to our department during 1 1/2 years and did not suffer from other disorders than the thyroid disease The diagnosis of hypo- or hyperthyroidism was established by the presence of typical clinical manifestations favourable response to therapy and was supported by appropriate laboratory abnormalities based on the reference value

Reprint requests to K Johansen Department of Internal Medicine and Endocrinology DK 2730 Herlev Denmark

Abbreviations T_3=triiodothyronine T_4=thyroxine FT_3I=free T_3 index FT_4I=free T_4 index TSH=thyrotropin BMR=basal metabolic rate

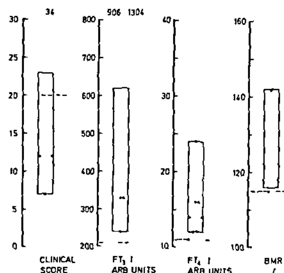


Fig 1 Clinical score index, FT_3I FT_4I and BMR in hyperthyroidism =Upper limits of the reference values Rectangles=mean±2 S D

(mean ± 2 S D) of our laboratory In the hypothyroid subjects 3 of the following criteria were fulfilled serum $FT_4I \leq 4$ 4 serum $FT_3I \leq 130$ arbitrary units BMR <90% and serum TSH ≥5 μU/ml In the hyperthyroid patients 2 of the following criteria were fulfilled serum $FT_4I > 11$ serum $FT_3I > 210$ arbitrary units and BMR >115% Clinical status was later assessed by a single observer with no knowledge of the BMR and the biochemical data It was scored in the thyrotoxic patients according to the index of Crooks et al (2) Scores of ≥20 suggest definite thyro-

of ≤10 definite non toxicosis and of 11–19

cases In the myxoedematous patients the clini

status was assessed by the index of Murray (13)

of ≥5 suggest definite myxoedema and of <5

dism

The concentrations of free T_4 and T_3 were expressed as FT_4I and FT_3I using the product of the total hormone concentration and the T_3 resin uptake (4) Serum T_4 was determined by a modification of the Murphy method (14) serum T_3 (15) and TSH (17) by radioimmunoassay BMR was measured by conventional technique with determination of oxygen consumption in the resting patient after an overnight fast and expressed as a percentage of a standard value for persons of the same age sex and surface area using the Harris Benedict standards (3)

The between assay coefficients of variation in the hypothyroid range were 26 20 17 10 and 4% on the determinations of serum T_4 T_3 TSH T_3 resin uptake and BMR respectively In the hyperthyroid range the coefficients were 6 6 5 and 3 on the serum T_4 T_3 T_3 resin uptake and BMR measurements

Total and partial correlation analyses were performed according to conventional statistical procedures (1) The 5% limit was accepted as indicating statistical significance

RESULTS

Fig 1 shows the clinical score BMR FT_3I and FT_4I in the hyperthyroid patients Fig 2 shows the same parameters and serum TSH concentrations in the hypothyroid patients

The results of the correlation analyses are summarized in Table I In hyperthyroidism the clinical score was positively correlated to both FT_4I and FT_3I The clinical score was negatively correlated to FT_3I and FT_4I in hypothyroidism The clinical score was correlated to BMR in both hyper and hypothyroid patients The serum TSH concentration in the hypothyroid patients was not correlated to the clinical score The correlations between clinical scores and FT_4I and FT_3I dropped to insignificant levels when the interdependence between the FT_4I and FT_3I was taken into account FT_4I was significantly correlated to FT_3I in both hyper and hypothyroid subjects

DISCUSSION

Four (14%) of the hyperthyroid patients had a FT_4I which could be described as borderline Borderline phenomenon was less common for the FT_3I (4%), confirming the diagnostic superiority of the serum T_3 concentration in hyperthyroidism (5 10 11) Six (21%) of the hyperthyroid patients had a BMR of ≤115%, indicating a less predictive role of BMR compared to FT_4I and FT_3I for the

Table I *Total and partial regression analyses*

1=Clinical score 2=FT_3I 3=FT_4I 4=BMR 5=serum TSH

	Thyrotoxicosis (N=28)		Myxoedema (N=13)	
	r	$p<$	r	$p<$
r_{12}	+0 53	0 01	−0 79	0 01
r_{13}	+0 47	0 05	−0 80	0 01
r_{14}	+0 42	0 05	−0 62	0 05
r_{23}	+0 69	0 001	+0 77	0 01
r_{24}	+0 45	0 05	+0 64	0 05
r_{34}	+0 41	0 05	+0 45	N S
$r^a_{12.3}$	+0 33	N S	−0 44	N S
$r^a_{13.2}$	+0 16	N S	−0 48	N S
r_{15}	–	–	−0 14	N S

[a] Partial correlation analyses taking into account the interdependence of FT_3I and FT_4I

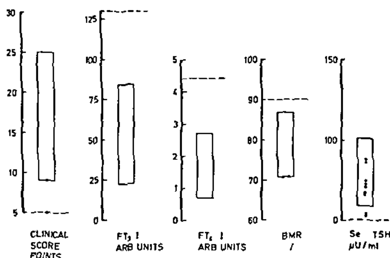


Fig 2 Clinical score index FT3I FT4I BMR and serum TSH in hypothyroidism =Lower limits of the reference values (upper limit of the clinical score index) Rectangles=mean±2 S D

diagnosis of hyperthyroidism Eight (28%) of the thyrotoxic subjects according to the definition had an elevated and eight (28%) a normal clinical score Twelve (43%) of the patients had scores in the equivocal range

In the hypothyroid subjects there was no difference in the predictive values of FT_3I and FT_4I and no borderline values existed BMR was abnormally low in 10 of the 11 patients and the serum TSH concentrations were elevated in all We thus failed to demonstrate any significant difference in diagnostic validity between serum TSH BMR FT_3I and FT_4I The clinical score was borderline in one patient and elevated in the others

Crooks et al (2) found significant correlations although weak in thyrotoxic subjects between the diagnostic index score and BMR ($r=+0\ 33$) 4 hour uptake of radioiodine ($r=+0\ 36$) and the values for 48 hour protein bound plasma radioactivity ($r=+0\ 36$) Mortimer et al (12) failed to find a significant correlation between pretreatment clinical score and FT_3I and FT_4I in thyrotoxic subjects However when pretreatment results and those during the first 12 weeks of carbimazole treatment were considered together a curvilinear relation was obtained Correlation coefficients were not presented

In our hyperthyroid patients no difference existed in degree of correlation between the clinical score on the one hand and the FT_3I and FT_4I on the other Both correlations were statistically significant Statistically significant correlations were also obtained in hypothyroid patients between the clinical score and the FT_3I and FT_4I and again no difference was seen in degree of correlation (Table I)

The significant correlations between clinical scores and the FT_3I and FT_4I dropped to insignificant levels in both hyper and hypothyroidism when the interdependence between the FT_3I and FT_4I was taken into account Serum TSH level was not correlated to the clinical state in the present study

We have found that the log serum TSH concentration is significantly and negatively correlated to both FT_3I and FT_4I in myxoedematous patients (7)

Determinations of BMR have been widely used as a measure of the thyroid hormone activity at tissue level The degree of correlation between the clinical score index and the thyroid hormones is however higher than between the thyroid hormones and BMR (Table I) The clinical score index thus appears to be a better index of severity of myxoedema and thyrotoxicosis than BMR

We have previously shown that the T_3 level in blood reflects the height of BMR better than the T_4 level (6) The present study shows that the clinical state of overt hyper or hypothyroidism is significantly correlated to the concentration of both T_4 and T_3 in blood and that the two hormones make equal contributions to the control of the clinical manifestations

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TSH Response Pattern to TRH Test and Optimum Time of Blood Sampling in Sporadic Euthyroid Goitre

Mogens Blichert Toft Claus Christiansen Christen K. Axelsson and Jørgen Egedorf

From Surgical Department D and the Department of Clinical Chemistry Glostrup Hospital Copenhagen Denmark

ABSTRACT Attempts were made to estimate the response pattern of thyrotrophin (TSH) to thyrotrophin releasing hormone (TRH) and the optimum time of blood sampling in patients with sporadic euthyroid goitre Of the 65 subjects studied 21 served as a reference group and 44 were patients with sporadic euthyroid goitres divided into diagnostic subgroups according to type of goitre Patients with a single autonomous thyroid adenoma were excluded The classification of goitre was based on clinical features, thyroid function tests, thyroid imaging studies using ^{99m}Tc pertechnetate uptake and examination of thyroid specimens originating from selective goitre resection carried out after the laboratory Investigations A standardized i v TRH test was performed in all probands and the TSH response was followed for 60 min postinjection There was a definite trend towards lack of response with increasing nodularity of the thyroid gland Moreover, no further information concerning the TSH response pattern was achieved by extending the blood sampling period beyond the +20 min limit of the time axis The study lends support to the view that increasing functional autonomy is a general trend in goitre evolution

Patients with sporadic euthyroid goitre do not seem to develop a uniform response pattern of thyrotrophin (TSH) to thyrotrophin releasing hormone (TRH) It is now well established that in patients with a solitary autonomous nodule the TSH response to TRH is absent or considerably decreased even before overt hyperfunction is evident (4 6 9 16) Less appreciated however is an apparently marked heterogeneous response pattern in other forms of sporadic euthyroid goitres indicating varying degrees of TSH dependency

It has long been held that goitre maintenance is TSH-dependent This view is supported by a normal or increased serum TSH level and TSH response to TRH in euthyroid goitrous patients (2 8 11 13 14 15 17 20) Recently however this concept has been challenged by the demonstration of a decreased serum TSH level and a blunted or severely impaired TSH response to TRH in a considerable proportion of patients with euthyroid nodular goitre though not including the single autonomous nodule (5 7 10 19)

This study lends support to the view that goitre maintenance may be TSH independent A heterogeneous response pattern of TSH to TRH in euthyroid goitres was demonstrated and the optimum time of blood sampling established

STUDY POPULATION AND METHODS

The study comprised 65 subjects Twenty one the reference group were healthy individuals with no history of thyroid diseases 44 were referred to the Surgical Unit due to sporadic euthyroid goitre Sex age and goitre classification are given in Table I None received any medication including hormonal contraceptives The patient group comprised a consecutive series studied during a one year period

Thyroid function was determined in all participants by measuring total serum thyroxine (T_4) total serum triiodothyronine (T_3) serum TSH and T_3 resin uptake The median value and range for each group are given in Table I In the patients a ^{99m}Tc pertechnetate scintigraphy of the thyroid gland was performed systematically at the Department of Clinical Physiology Finally i v TRH stimulation test was performed in all probands Excluded were patients with a single autonomous nodule verified by T_3 suppression test followed by TSH stimulation test and repeated scans

Laboratory methods

Serum T_4 was measured by competitive protein binding procedure (Tetrasorb Abbott) and serum T_3 by a

Abbreviations TSH = thyrotrophin ΔTSH = TSH response TRH = TSH releasing hormone T_3 = triiodothyronine T = thyroxine

Table I *Clinical and laboratory findings in 21 normal subjects and 44 patients with sporadic euthyroid goitre (median and range)*

	N	Age (y)	Serum T_4 (nmol/l)	Serum T_3 (nmol/l)	T_3 resin uptake index	Serum TSH (mU/l)
Normal subjects						
Females	10	30 (21–39)	91 (80–127)	2 25 (1 55–2 85)	101 (84–109)	2 9 (≤1 5–4 0)
Males	11	29 (24–39)	90 (66–115)	2 18 (2 05–2 80)	105 (97–121)	2 3 (≤1 5–3 6)
Euthyroid goitres						
Diffuse						
Females	4	33 (20–47)	107 (86–129)	2 20 (2 15–3 05)	96 (87– 97)	2 2 (≤1 5–3 1)
Uninodular						
Females	13	41 (23–80)	102 (85–126)	2 35 (1 60–2 90)	97 (84–119)	1 6 (≤1 5–3 0)
Males	5	37 (23–57)	107 (92–135)	2 15 (2 05–2 65)	95 (92–110)	≤1.5 (≤1 5)
Multinodular	19	51 (27–76)	110 (79–144)	2 60 (1 95–3 45)	94 (80–122)	≤1 5 (≤1 5–3 4)
Females						
Males	3	56 (53–56)	120 (101–121)	2 45 (2 20–3 00)	90 (89– 96)	≤1 5 (≤1 5–2 4)

radioimmunoassay (Abbott) Serum TSH was measured by radioimmunoassay technique using the kit from Pharmacia Diagnostics T_3 resin uptake (Triosorb Abbott) values were expressed proportionally in relation to a normal reference serum fixed at 100 Reference values for the methods employed were based on results obtained in our laboratory for 103 normal healthy individuals 56 female and 47 male (age range 21–69 years) For serum T_4 the reference range was 58–173 nmol/l (median 100) for serum T_3 1 5–3 6 nmol/l (median 2 4) and for T_3 resin uptake index 67–129 (median 98) For serum TSH the reference range was ≤1 5–4 3 mU/l (median 2 0) indicating a detection limit of 1 5 mU/l The range is signified by the lowest and highest values measured No significant sex or age related difference was recorded in T_4 T_3 and TSH values

cification of goitres

ll 44 patients with sporadic goitre were unequivocally assured euthyroid both clinically and biochemically (Table I) Goitre classification was based on palpatory findings and further supported by thyroid imaging studies using ^{99m}Tc uptake In the subgroup classified as diffuse goitre (4 patients) the ^{99m}Tc uptake was regular In uninodular goitres (18 patients) a circumscribed defect (cold nodule) was visualized on the scintigram corresponding to the palpable mass on the neck whereas in the

Table II *Serum TSH response (mU/l) after TRH stimulation in the reference group*

	20 min	30 min	40 min	60 min
Females (n=10)				
Median	7 4	6 8	6 1	4 0
Range	3 9–17 0	3 2–14 4	2 6–12 5	1 1–8 3
Males (n=11)				
Median	3 8	4 3	3 2	3 1
Range	2 5–5 9	1 8–5 9	1 4–5 2	1 3–4 5

subgroup with multinodular goitres (22 patients) the ^{99m}Tc uptake was irregular with scattered non functioning areas (cold areas) The assignment to subgroups agreed with gross findings at subsequent surgical treatment

TRH test

Synthetic TRH (Hoechst) 200 μg i v was injected rapidly between 9 and 10 a m Blood was sampled prior to (−5 and 0 min) and 20 30 40 and 60 min postinjection for determination of TSH ΔTSH was defined as the rise in serum TSH concentration beyond the 0-value

RESULTS

Table II shows the TSH responses (ΔTSH) to TRH in the reference group All 21 probands responded to TRH and presented a smooth response curve The peak value was attained at 20 min postinjection except for 2 females and 4 males who reached the peak at +30 min However the difference between values at +20 and +30 min was slight varying from 25 to 1% of the peak median 6% The peak was followed by a gradual decline The TSH response was significantly higher in females than in males (p<0 05)

The TSH responses to TRH are plotted in Fig 1 along the y axis against time for each patient The absolute values of the reference range at each sampling point and for each sex are given in Table II

Patients with diffuse euthyroid goitre showed a completely normal response pattern whereas those with nodular goitre displayed a definite trend towards lack of response with increasing nodularity (Fig 1) In 2 patients with uninodular goitre an exaggerated response was found while in 24 pa

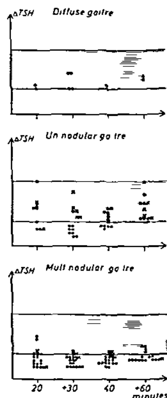


Fig 1 Individual TSH response (*ΔTSH*) to TRH test in 44 patients with sporadic euthyroid goitre expressed proportionally to corresponding ΔTSH values derived from reference groups Hatched area indicates the reference range marked out as a fixed interval irrespective of sex and sampling time × = Males ● = females

tients (54%) the TSH response to TRH was either blunted or impaired Decreased response occurred predominantly in the subgroup with multinodular goitres Further it was a general feature that the outcome of the test response—whether increased decreased or normal—at one sampling point was valid for the outcome at the other sampling points No point on the time axis was superior in separating thyroid functional normality and abnormality as demonstrated by a pronounced overlapping of ΔTSH ranges at all points of sampling in all groups (Fig 1)

Analogous calculations with the raw serum TSH values during TRH test instead of ΔTSH values showed exactly the same pattern

In patients responding to TRH the peak level was attained at +20 min except for 3 patients with uninodular goitre who all reached the peak at +30 min The peak was followed by a gradual fall of TSH levels In TRH unresponsive patients (ΔTSH≤1 mU/l) the +20 min value expressed the final test result indicating that delayed response was not observed

DISCUSSION

This study was designed to describe the TSH response pattern to TRH in sporadic euthyroid goitres among which the single autonomous adenoma was excluded and further to estimate the optimum time of blood sampling during the test

We observed a marked heterogeneous response pattern of TSH to TRH with a definite trend towards lack of response with increasing nodularity of the thyroid gland This finding most likely indicates an increasing functional autonomy in goitre evolution Our results are in complete agreement with those most recently described by Gemsenjager et al (7), Dige Petersen and Hummer (5) and Kirkegaard et al (10) In their studies 20 21 and 33% of patients respectively had impaired or absent TSH response to TRH compared with 54% in our series The fact that our patients were a surgical series with relatively large goitres and a predominance of multinodular forms of long duration may well explain the discrepancy in frequency That goitrous tissue may show increasing autonomous behaviour is supported by autoradiographic studies (18) and scintigraphic findings demonstrating subnormal suppressibility of the goitrous gland by T_3 (3 10 12) From two of these studies (3 12) it also appears that autonomously functioning tissue may not be confined to nodules but may show a variable internodular distribution as well This may explain our finding of TRH refractoriness in uninodular goitres with exclusively cold nodules on the scintigram The observation that TSH responsiveness to TRH recovered after selective goitre resection without biochemical evidence of thyroid hypofunction lends further support to the theory of increasing functional autonomy as a general trend in goitre evolution (1 7)

The optimum time of blood sampling for determination of the TSH response to TRH in sporadic euthyroid goitres has not been investigated in detail In particular it is not known whether the time reflecting maximum serum TSH response to TRH also represents the optimum time for differentiating functional thyroid abnormality from normality In nearly all responders among our patients the peak

TSH level was attained at +20 min consistent with the pattern in normals Moreover the diagrams clearly show that no further information concerning the ΔTSH pattern was achieved by extending the sampling period beyond the +20 min limit of the time axis whether or not a response was obtained In all subgroups a marked overlap in ΔTSH values was evident at any sampling point Hence the +20 min values were not inferior to those derived from the other sampling points in describing the response pattern in sporadic euthyroid goitre Therefore we consider the sampling point at +20 min to be the optimum time of blood sampling

It is apparent from this study that increasing functional autonomy develops parallel with increasing nodularity of the thyroid gland Moreover, the TRH test followed for 20 min seems to offer an appreciable improvement in diagnosing subclinical functional abnormalities in sporadic euthyroid goitres

ACKNOWLEDGEMENT

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Clinical Value of Total T_4 and T_3 Determinations in Patients with Suspect Hyperthyroidism before and after Correction for Binding Proteins

Gunnar Kallner Bertil Kågedal Jan Gustaf Ljunggren and Monica Tryselius

From the Departments of Medicine St Gorans Hospital and Sodersjukhuset Stockholm and the Department of Clinical Chemistry University of Linkoping Linkoping Sweden

ABSTRACT In a previous investigation we found that the determination of serum T_3 by a radioimmunoassay procedure was superior to both the determination of serum T_4 and the TRH test for laboratory discrimination between eu and hyperthyroidism The investigation comprised 50 patients in whom hyperthyroidism could not be excluded by the first clinical examination alone The complete investigation showed that 26 of the patients had hyperthyroidism and 24 had normal thyroid function The aim of the present study was to elucidate whether the discriminatory power of T_3 and T_4 estimations in these patients could be increased by correction for variations in the hormone binding proteins The influence of the proteins was estimated by the T_3 uptake test and determination of the TBG level by radioimmunoassay technique The free T_4 and T_3 indices were calculated according to standard procedure The results demonstrate that all subjects had TBG values within the reference levels In the discrimination between normal and hyperthyroid states, the free T_4 index gave more reliable results than the uncorrected T_4 or the T_4 TBG ratio However, the discriminatory power of T_3 was superior to both T_4, T_4 TBG ratio and free T_4 index No improvement was obtained by calculation of the T_3 TBG ratio A slight improvement was obtained by calculation of the free T_3 index It is concluded that estimation of the total serum T_3 level is superior to the total serum T_4 level in the laboratory discrimination between eu and hyperthyroidism even subsequent to correction for the binding proteins

Key words Triiodothyronine thyroxin free T_4 index free T_3 index thyroid function hyperthyroidism

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Disorders of thyroid function are commonly encountered in clinical medicine The diagnosis of these conditions is based upon a detailed medical history a full physical examination and laboratory tests In the last few decades many different tests have been developed (4) However it was not until the recent development of radioimmunoassay techniques that a sufficiently sensitive and simple means of direct estimation of the hormone levels has been possible in both normal and pathological states (9) The rapid increase in laboratory costs for the various analyses has emphasized the need for proper selection of tests especially with regard to initial procedures

It is well recognized that the determination of serum thyroid stimulating hormone (TSH) levels by radioimmunoassay is a reliable test of primary hypothyroidism (6) In fact a normal TSH level excludes the diagnosis It is not as clear however as to which tests should be used in the first evaluation of patients with possible thyroid hyperfunction In a recent investigation into the laboratory discrimination between eu and hyperthyroidism using radioimmunoassay techniques we demonstrated that estimation of the total levels of triiodothyronine (T_3) was superior to estimation of the total levels of thyroxine (T_4) (14) Both tests exhibited better discriminatory power than the thyrotrophin releasing hormone (TRH) test although a normal response to TRH excluded hyperthyroidism However it was not investigated as to whether the discriminatory power of the tests could be further increased by correcting for possible variations in the hormone binding proteins Therefore the purpose of the present investigation was to elucidate this problem

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Abbreviations TSH=thyroid stimulating hormone TRH=thyrotrophin releasing hormone TBG=thyroxine binding globulin T_4=thyroxine T_3=triiodothyronine T_3U=T_3 uptake

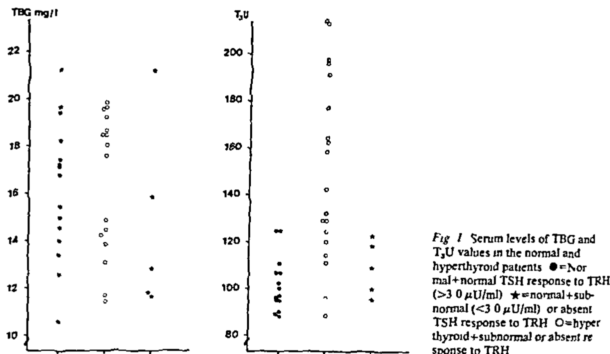


Fig 1 Serum levels of TBG and T_3U values in the normal and hyperthyroid patients ●=Normal+normal TSH response to TRH (>3 0 μU/ml) ★=normal+subnormal (<3 0 μU/ml) or absent TSH response to TRH O=hyperthyroid+subnormal or absent response to TRH

STUDY POPULATION AND METHODS

Patients and subjects studied have been described in a previous investigation (14) Due to lack of serum six of the 26 normal subjects were excluded from the present investigation All six showed T_4 and T_3 concentrations within the reference limits For the same reason five of the 24 hyperthyroid patients were excluded They all demon increased concentrations of T_3 and T_4 except one showed a T_4 concentration within the reference in

The serum concentrations of T_4 and T_3 were determined by radioimmunoassay (11) The reference interval (±2 S D) for T_4 was 54–124 nmol/l and for T_3 1 1–2 5 nmol/l *for subjects aged 15–60 years The T_3 uptake (T_3U) test* was performed using Sephadex as adsorbent (12) The reference interval was 80–120 % of the pooled serum from healthy individuals The free T_4 index (3) was calculated as $(T_4)\times(T_3U)/100$ and the free T_3 index as $(T_3)\times(T_3U)/100$ The serum concentration of thyroxine binding globulin (TBG) was determined by radioimmunoassay (8) The reference interval was 10 1–21 5 mg/l

The statistical evaluation was performed by Dr Ekblom Department of Statistics University of Stockholm

RESULTS

Fig 1 shows the TBG concentration and the T_3U in the normal and hyperthyroid groups It can be seen that all the hyperthyroid patients showed TBG concentrations within the reference interval Although 14 of the 19 hyperthyroid patients had T_3U well above the upper reference value the test is not suitable by itself as a discriminant between the two groups

Fig 2 shows the T_4 concentrations, the free T_4 index and the T_4 TBG ratio in the normal and the hyperthyroid patients Two of the 20 normal subjects had T_4 concentrations slightly above the upper reference value Their hormone values were T_4 136 and 148 nmol/l T_3 2 0 and 1 6 nmol/l T_3U 89 and 102 % TBG 21 3 and 19 7 mg/l respectively Their increased T_4 concentration could thus be explained by a rather high TBG concentration and calculation *of free T_4 index and T_4 TBG ratio gave quite nor*mal values in one of them Fig 2 also shows that the hyperthyroid patients compared to the normals demonstrated a 3 fold increase in the mean free T_4 index *as compared to the 2 fold increase in the* mean T_4 values Three of the hyperthyroid patients with T_4 within the reference interval exhibited an increased free T_4 index One hyperthyroid patient with an increased T_4 of 138 nmol/l had a free T_4 index of 121 nmol/l When the values for T_4 TBG ratio around an arbitrarily chosen limit of 7 0 were considered two normal subjects showed increased values and four hyperthyroid patients showed normal values Thus in discrimination between the normal and hyperthyroid state it seems that the free T_4 index may give more reliable results than the uncorrected T_4 or the T_4 TBG ratio

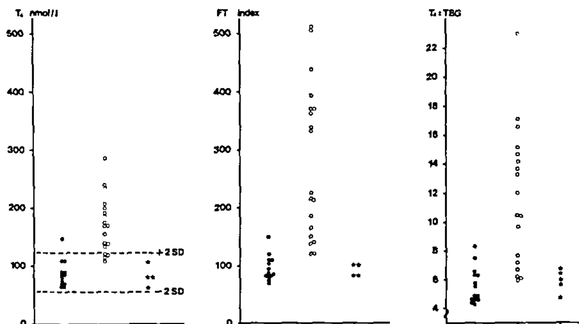


Fig 2 Serum levels of T_4 free F_4 (FT_4) index and T_4 TBG values in the normal and hyperthyroid patients Symbols as in Fig 1

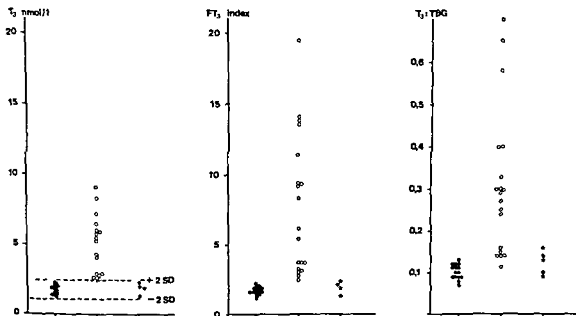


Fig 3 Serum levels of T_3 free T_3 (FT_3) index and T_3 TBG values in the normal and hyperthyroid patients Symbols as in Fig 1

Table I *Hormone concentrations and derived parameters in the normal and hyperthyroid groups (mean ±S D)*

	Normal group (n=20)	Hyperthyroid group (n=19)
TBG (mg/l)	16 2±3 2	15 8±3 3
T_3U (%)	104 7±12 3	150 4±39 2
T_4 (nmol/l)	90 1±23 0	177 3±51 8
Free T_4 index (nmol/l)	93 0±20 0	275 2±136
T_4 TBG ratio	5 63±1 13	11 91±4 67
T_3 (nmol/l)	1 73±0 36	4 74±2 09
Free T_3 index (nmol/l)	1 77±0 29	7 69±4 98
T_3 TBG ratio	0 108±0 022	0 326±0 189

Fig 3 shows the T_3 concentrations the free T_3 index and the T_3 TBG ratio in the normal and hyperthyroid groups The hyperthyroid patients had a more pronounced increase in free T_3 index than in the T_3 values as compared to the normals The hormone concentrations for the hyperthyroid patient with the T_3 concentration within the reference interval were T_3 2 2 nmol/l T_4 120 nmol/l T_3U 114% and TBG 19 7 mg/l The calculated free T_3 index of 2 5 was at the upper reference level and the free T_4 index was slightly increased 137 nmol/l As seen from Fig 3 no improvement in the separation of the two groups was obtained by calculating the T_3 TBG ratio when an arbitrary limit of 1 35 as chosen as the quotient

he hormone concentrations and derived pa meters in the euthyroid and hyperthyroid groups re summarized in Table I The differences between the groups were significant (p<0 001) for all variates except the TBG concentration

In conclusion the results demonstrate that total T_3 estimation was superior to total T_4 estimation even after correction for the influence of the hormone binding proteins in the laboratory discrimination between eu and hyperthyroidism

DISCUSSION

The thyroid hormones T_4 and T_3 circulate both as free hormones and as bound complexes to specific serum proteins It is generally recognized that the free fractions are the biologically active components and the bound fractions are inert It has thus been logical to assume that determinations of the free thyroid hormone concentrations in serum comprise the methods of choice in the laboratory diagnosis of thyroid function Recently a relatively simple radioimmunoassay technique was described for the direct measurement of the free hormone levels in serum dialysate (5) However its clinical usefulness was evaluated (15) and it was concluded that the free hormone determination was unlikely to play a major role in the routine investigation of thyroid disease One reason for this conclusion was the statistically significant correlation found between the free hormone levels and their respective total hormone levels and that the estimations of the free levels were more complicated than the estimation of the total level

Determination of the total concentrations of T_4 and T_3 by radioimmunoassay techniques has been shown to be a simple and reliable method suitable for routine clinical use However, the total hormone levels are influenced by variations in the concentrations of the hormone binding proteins particularly TBG TBG concentrations may vary under the influence of many different conditions and factors such as pregnancy liver and kidney diseases genetic factors drugs and hormones (13)

During the last few decades several tests have been described for an indirect evaluation of the amount of circulating TBG e g the T_3 resin uptake test Recently immunoassays have been developed for the direct determination of TBG which may be more adequate than the indirect techniques Thus it has been demonstrated (2) that correction of serum total T_4 concentration according to the actual TBG concentration T_4 TBG ratio provided a better correlation with the thyroid state than the free T_4 index

Our present results indicate that total T_3 estimation has even better discriminatory ability between normal and hyperthyroid subjects when compared to total T_4 estimation T_4 TBG ratio or FT_4 index It must however be noted that all our subjects had normal TBG levels It is thus unknown whether similar conclusions can be drawn concerning patients with abnormally high or low TBG levels The normal levels of TBG in our hyperthyroid group confirm recent results (7) Earlier investigations had indicated lower TBG levels in hyperthyroid patients than in normals

It can be discussed whether or not T_4 determination should be included in the first measurements for discrimination between normals and hyperthyroid patients A firm conclusion cannot be made until further studies are carried out on pa

tients with abnormally high or low TBG levels It must also be established whether conditions such as serious illnesses which are found to reduce the T_3 but not the T_4 levels despite a normal thyroid function also affect the T_3 levels in hyperthyroid patients under similar conditions (10) If a similar reduction is found in hyperthyroid patients false low T_3 levels would be obtained Finally it must also be conclusively demonstrated whether T_4 toxicosis exists (1)

In conclusion the results of the previous (14) and the present investigation show that total T_3 estimation by immunoassay is a suitable test for the initial laboratory discrimination between normal and hyperthyroid patients If the T_3 level is close to the upper normal limit T_3U or TBG estimation may be useful If there is any doubt about the diagnosis after clinical investigation and laboratory tests a TRH test should be performed A normal TSH response to the TRH test excludes hyperthyroidism An impaired or absent response can occur both in normal and hyperthyroid patients In such cases hyperthyroidism must be excluded by other means e g T_3 suppression test

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Ventricular Extrasystoles and Intracellular Electrolytes in Hypokalemic Patients before and after Correction of the Hypokalemia

Thomas Dyckner and P O Wester

From the Department of Medicine Serafimerlasarettet Stockholm and the Department of Medicine University Hospital Umea Sweden

ABSTRACT Fifty four initially hypokalemic patients, 43 of whom were on diuretic treatment, were given potassium supplementation until they showed a repeatedly normal serum potassium level Muscle specimens obtained by percutaneous biopsy revealed that there were no concomitant increases in muscle potassium content, nor in intracellular potassium concentration, except in the very small group (6 patients) with a muscle magnesium content of ≥3 95 mmol/100 g fat free dry solids (FFDS) and an initially lower muscle potassium content (≤39 9 mmol/100 g FFDS) ECG, registered for 3 hours on a portable ECG tape recorder before and after correction of the serum potassium level, showed no change in the frequency of ventricular ectopic beats

Key words hypokalemia potassium therapy intracellular potassium ventricular ectopic beats
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The treatment with most diuretics induces an increased renal loss of potassium which is well known but there is also an increased loss of magnesium in the urine (10 14 25) The potassium losses are usually compensated for by oral potassium supplementation but the magnesium losses have been neglected In an earlier investigation on patients with hypokalemia and/or on diuretic treatment (11) we found that the muscle content of magnesium (Mg/m) influences the correlation between serum potassium (K/s) and muscle potassium content (K/m) the correlation being higher when patients with a low Mg/m were omitted

The aim of the present study was firstly to investigate K/m and Mg/m in patients with hypokalemia and to study the effects of normalization of K/s on K/m and Mg/m and secondly to assess the frequency and type of ventricular ectopic beats (VEBs) before and after the correction of the serum potassium level

STUDY POPULATION

In an earlier study on the relation between extra and intracellular electrolytes in patients with hypokalemia and/or diuretic treatment 107 patients were investigated (11) The present study comprises 54 of those patients 21 men and 33 women (mean age 63 1±11 0 and 69 4±10 6 years respectively) chosen on account of hypokalemia (<3 5 mmol/l) At the time of the first muscle biopsy 1–2 days later however 13 patients had a normal K/s

Twenty six patients had congestive heart failure with basal pulmonary rales and apical vascular enlargement on chest X ray 17 were being treated for arterial hypertension and 9 suffered from liver diseases mainly on an alcoholic basis with raised levels of s ASAT and s ALAT on admission One patient had diabetes mellitus and one vomited for unknown reasons

S-creatinine values of ≤120 μmol/l were found in 38 patients 121–200 μmol/l in 11 and ≤201 μmol/l in 5

Forty three patients were on and 11 were not on diuretic treatment Of the patients on diuretics 13 had been on this treatment for less than 3 months 11 for 3 months – 3 years and 19 for more than 3 years

METHODS

Blood samples for estimation of Na K Mg s-creatinine total carbonate and digitalis concentrations were taken from all patients on admission The hypokalemic patients were investigated by percutaneous muscle biopsy ad modum Bergström (3) on the following morning after an

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Abbreviations VEBs=ventricular ectopic beats FFDS=fat free dry solids Mg/m=muscle magnesium Mg/s=serum magnesium K/s=serum potassium K/m=muscle potassium K/ic=intracellular potassium K/ec=extracellular potassium H_2O/ic=intracellular water H_2O/ec=extracellular water H_2O/m=muscle water Na/m=muscle sodium Na/ic=intracellular sodium Cl/m=muscle chloride

Table I *Muscle extra and intracellular values for electrolytes and water before and after correction of K/s (mean ± S D)*

Amounts in mmol/l (serum values) mmol/100 g FFDS (muscle values) mmol/kg intracellular water (intracellular values) water expressed in g/100 g FFDS

	K/s	Mg/s	H_2O/ec	K/m	Mg/m	Na/m	Cl/m	H_2O/m	K/ic	Na/ic	H_2O/ic
Before K	3 40± 062	0 79± 0 15	124± 75	40 4± 4 35	4 01± 0 35	20 5± 9 33	15 2± 9 13	401± 67	146± 17 7	16 9± 12 1	277± 40
After K	4 27± 0 53	0 82± 0 16	142± 72	39 7± 5 88	3 81± 0 47	24 2± 10 5	19 6± 11 4	418± 65	148± 19 6	17 0± 11 4	267± 48

overnight fast Simultaneously blood samples were again obtained for determination of Na K Mg Cl s creatinine total carbonate and digitalis concentrations After the muscle biopsy a 3 hour ECG tape recording was made (Avionics 12h tape recorder) and the frequency and type of VEBs were registered The tapes were read by one of the authors VEBs were defined as QRS complexes occurring too early with a duration exceeding 0 10 sec without a preceding P wave and with a configuration differing from the usual QRS complexes To classify the VEBs further the traditional classification of Lown et al (16) was used

Following these procedures the patient was given potassium supplementation either per os or i v in sufficient amounts to raise the serum potassium level constantly to normal values In most cases this took about 4 days When K/s was repeatedly within normal limits a new muscle biopsy was performed in the morning after an overnight fast and simultaneously a new set of blood samples was taken for the same analyses as before the first muscle biopsy Thereafter a new 3 hour ECG tape recording was made for assessing the frequency and type of EBs following the normalization of K/s

The muscle biopsies (40–80 mg wet weight) were rapidly issected free from all visible fat and connective tissue nd rolled on a piece of quartz glass to remove all traces of blood The muscle tissue was then attached to a preweighed platinum hook and repeatedly weighed on a Cahn 4700 electrobalance The original wet weight was obtained by extrapolation to zero time The platinum hook with adhering muscle tissue was then placed in an oven at 110°C till constant weight to obtain the dry weight and the water content of the specimen Fat was then extracted with redistilled petroleum ether After a new drying period the fat free dry solid (FFDS) weight was obtained

The muscle tissue was then wet ashed in 1N nitric acid and the electrolytes were determined by atomic absorption spectrophotometry (Varian Techtron 1100) in the solution left according to methods elaborated by Bergstrom et al (5) Chloride was determined by atomic absorption spectrophotometry after precipitation with silver nitrate (11) The intracellular electrolyte concentrations were determined according to the chloride method (13) assuming chloride is freely diffusible across the cell membrane A normal resting membrane potential of 87 2 mV was assumed (6)

Serum electrolytes were determined by flame photometry (Na K) atomic absorption spectrophotometry (Mg) autoanalyzer technique (total carbonate and s-creatinine) and by titration with silver nitrate (Cl) Protein was determined by the biuret method Digitalis concentration was determined by radioimmunoassay method (24)

Paired *t* statistics were used to compare the electrolyte values and the frequency of VEBs before and after the correction of K/s

RESULTS

Table I shows the muscle the extra and intracellular values for electrolytes and water in the whole patient series before and after correction of K/s It is quite obvious that there is no appreciable change in K/m or K/ic after correction of K/s despite the large increase in K/s Even when the 13 normokalemic patients are omitted there is still no increase in K/m or K/ic after correction of K/s

Dividing the material according to diagnoses treatment with digitalis or not s creatinine level or toal carbonate values does not result in any significant increase in K/m or K/ic after correction of K/s Neither is there any statistically significant increase in these parameters after correction of K/s in the group with a Mg/m of ≥3 95 mmol/100 g FFDS In this group however there were many patients with an initial K/m of ≥40 0 mmol/100 g FFDS and if only the patients with a

Table II *Number of patients in the different VEB groups before and after correction of K/s*

VT=Ventricular tachycardia VF=ventricular fibrillation

	No of VEBs/h				VT VF
	0	1–60	61–300	>300	
Before K	11	25	6	9	3
After K	15	20	11	5	3

Table III *Changes in number of patients exhibiting certain types of VEBs before and after correction of K/s*

	Type of VEB						
	None	Occasional uniform	>5 uniform/min	Multi form	Repetetive bigeminal	R on T	Ventricular tachycardia or fibrillation
Before K	11	20	2	4	5	9	3
After K	15	16	2	7	5	6	3

Mg/m of ≥3 95 and a K/m of ≤39 9 mmol/100 g FFDS are taken into consideration there are significant increases in K/m and K/ic after correction of K/s even though the group is very small (6 patients) ($p<0$ 02 and $p<0$ 01 respectively)

The group of 15 patients with a lower Mg/m (≤3 94 mmol/100 g FFDS) and a lower K/m (≤39 9 mmol/100 g FFDS) shows an increase although not statistically significant in both K/m and K/ic after correction of K/s But K/m is still low in contrast to the group with a higher Mg/m

The patients on diuretics show no increase in K/m or K/ic after correction of K/s Dividing the patients according to the duration of treatment with diuretics shows that the group on diuretics for less than 3 months has a K/m of >40 0 mmol/100 g FFDS both before and after the correction of K/s The corresponding values in the group on treatment for 3 months – 3 years are slightly above 40 0 mmol/100 g FFDS before and slightly below this level after correction of K/s and in the group on diuretics for more than 3 years below 40 0 mmol/100 g FFDS both before and after correction of K/s Both before and after correction of K/s the K/m successively decreases when the period of treatment with diuretics increases None of the groups shows any significant changes in K/m or K/ic after correction of K/s

The ECG tape recordings reveal that there was no decrease in the frequency of VEBs after the correction of K/s nor were there any changes in the type of VEBs (Tables II and III) There are minor changes in the different VEB groups but the individual patient just as often goes from a less to a more serious group as the reverse Thus we did not observe any significant changes in the frequency or type of VEBs after correction of K/s The digitalis concentrations were unchanged before and after correction of K/s

DISCUSSION

Correction of K/s in our patients did not result in an increase in K/m or K/ic except in the very small group of patients with a higher Mg/m and an initially low K/m These six patients were 3 men and 3 women two with congestive heart failure two with arterial hypertension one with alcoholism and one who vomited for unknown reasons All had a normal s creatinine level except one who had a value between 121 and 200 μmol/l Only one patient was on digitalis Three patients were not on diuretics one had been on diuretics for less than 3 months one for 3 months – 3 years and one for more than 3 years

Magnesium is a necessary activator of Na–K–ATPase (23) which is crucial for the function of the sodium pump In the event of a magnesium deficiency the cell cannot attract enough potassium despite a normal serum potassium concentration This leads to disturbances in the extra/intracellular potassium balance The ratio between K/ec and K/ic is the main determinant of the resting membrane potential according to the formula

$$E = -61\ 5 \log \frac{K/ic}{K/ec}$$

A decrease in K/ic with an unchanged K/ec (≈K/s) will result in a less negative resting membrane potential This in turn will render the cell more easily excitable

Digitalis is an inhibitor of Na–K–ATPase which leads to the same result as a magnesium deficiency as far as the potassium balance is concerned As the treatment with most diuretics involves increased urinary losses of magnesium the combination of diuretics and digitalis seems especially dangerous

Some of the patients in the undivided series did

not have a low K/m but even when they were disregarded there was no increase in K/m after correction of K/s in any of the groups, except the one with a higher Mg/m This may be explained by the concomitantly low Mg/m in the other groups and hence the lacking activation of Na-K-ATPase

One has to consider whether the time available for the cellular uptake of potassium was too short in our study (range 3 days–3 weeks), thus invalidating the results Conway (8) has shown that the ingress of potassium is a very rapid process under normal conditions Therefore the potassium supplementation provided should have reached the intracellular space by the time of the second muscle biopsy

There are only a few, diverging investigations of whether potassium supplementations are retained by the body In 1951 Brown et al (7) found that oral potassium administration gave patients with heart disease a positive balance Nagant de Deuxchaisnes et al (20) in 1961 could not demonstrate an increase in total exchangeable potassium after potassium therapy Such an increase was obtained on the other hand by means of oral potassium supplementation by White (29) in 1970 in 7 patients with severe valvular heart disease McKenna et al (18) in 1971 in 7 patients and Edmonds and Jasani (12) in 1972 In 1977 MacLennan et al (19) could not, however raise the total body potassium in 13 elderly patients by oral potassium supplementation for 3 months The discrepant results of the above investigations reflect a different cellular magnesium content maybe a different frequency of medication with is between the groups

The question of whether treatment with diuretics induces a cellular potassium depletion has not been settled Most investigators have demonstrated a decrease in plasma potassium concentration and an increased urinary loss of potassium but the measurements of cellular potassium before and after diuretic treatment have been less conclusive Some authors have shown a decrease in exchangeable potassium (2 12 15 18 30) or in muscle biopsies (4) while others have not found any significant changes (1, 26) It should be noted however, that the latter investigations concerned patients whose diuretic treatment had been comparatively brief though in 1974 Dargie et al (9) found no evidence of depletion of total body potassium in 29 patients after treatment with frusemide for one year In our patients K/m and Mg/m decrease with increasing duration of diuretic treatment This favours the opinion that potassium and magnesium depletion gradually evolves during treatment with most diuretics

After correction of K/s there was no significant change in the frequency or type of VEBs In accordance with earlier studies on animals (27 28) it was possible to raise K/m in the small group with a higher Mg/m In this case Mg/m may have been sufficient to allow the cell to attract potassium through the action of the Na-K-ATPase In this very small group there were however, no significant changes in type or frequency of VEBs after correction of K/s

The duration of the ECG tape recording 3 hours was considered to be adequate for assessing a change in the frequency or type of VEBs before and after correction of K/s Ryan et al (22) have shown that a 1 hour printout of a 24 hour registration will result in the detection of 90–95% of VEBs present Lown et al (17) discovered 70% of VEBs during a 1 hour registration A 3 hour recording is of course unsatisfactory for detecting every arrhythmia that may occur in the single patient but this was not our intention

In conclusion our study implies that cellular potassium and magnesium decrease gradually in patients on treatment with most diuretics The potassium deficiency does not seem to be corrected unless magnesium is present in adequate amounts Potassium supplementation alone did not result in any change in the frequency or type of VEBs in our study In some studies it has been found that patients on diuretics have a higher frequency of VEBs than those not on diuretics (21) There is reason to consider whether these arrhythmias are secondary to the diuretic induced electrolyte disturbances between the extra and intracellular spaces

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Cardiovascular Risk Factor Changes in a Three-Year Follow-up of a Cohort in Connection with a Community Programme (the North Karelia Project)

Pekka Puska Jarmo Virtamo Jaakko Tuomilehto
Juhani Maki and Liisa Neittaanmaki

From the Co-ordinating Centre of the North Karelia Project National Public Health Laboratory Epidemiological Research Unit University of Kuopio Kuopio Finland

ABSTRACT A re-examination after 3 years was done in 1975 in a 20% random subsample (n=1683) of the representative population sample (males and females 25–59 years) that was examined in 1972 in North Karelia (NK), and a matched reference county as the baseline survey for the community programme in NK The changes in smoking habits serum cholesterol dietary fat consumption and systolic BP were more favourable among the subjects in the NK sample than among the reference sample, although the differences were generally small Results from multivariable analyses are presented to show the variables that predict a favourable risk factor change in the individual Living in NK is associated in the analysis with a favourable change in each of the three risk factors The limitation of this method in the evaluation of a community programme is discussed

Key words cardiovascular risk factors intervention community
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The North Karelia project was established after a petition from the local population for a comprehensive community programme for the control of cardiovascular diseases (CVD) in this county in Eastern Finland The mortality and incidence rates of CVD and especially coronary heart disease (CHD) are exceptionally high in this rural county with 180000 inhabitants (5)

The main objective of the programme is a decrease in the cardiovascular mortality and incidence among the North Karelian population with special reference to the middle aged and male population The intermediate objectives are to reduce the known CVD risk factors (smoking serum cholesterol blood pressure (BP)) among the population and to promote early detection treatment and rehabilitation of cardiovascular patients (2 3 4) Strong emphasis has been placed on primary prevention of the numerous disease attacks by a community action to influence the CVD related health behaviour of the population, i e by reducing smoking by changing the diet to lower the serum cholesterol level and by introducing antihypertensive drug treatment in the community to lower high BP (6 8 9 10)

The programme consists of a comprehensive community intervention The activities are integrated with the health services and social organization of the county Practical subprogrammes were developed in a systematic way following the natural course of CHD The subprogrammes have their practical objectives methods and inbuilt evaluation These consist of 1) delivery of health information through multiple channels 2) introduction of environmental changes (esp concerning smoking and diet) 3) organization of the services (reorganization of the basic health services and creation of necessary new services) 4) training of different groups of personnel involved in the programme and 5) necessary information services for the continuous evaluation

The aim of the evaluation is to assess the five year period 1972–77 concerning the feasibility effect and cost of the programme and the process that takes place in the community For the *main objectives* the basis for the evaluation includes mortality data hospital data and special myocardial in

Abbreviations CVD=cardiovascular diseases CHD=coronary heart disease BP=blood pressure

Table III *Percentages of current smokers among the cohorts in 1972 and 1975*

	North Karelia			Reference county		
	Urban	Rural	Total	Urban	Rural	Total
Males						
1972	45	47	47	44	47	46
1975	33	37	36	40	40	40
Females						
1972	16	8	10	9	6	7
1975	14	6	8	12	8	10

subjects at the baseline (1972) and after three years (1975) The tables indicate whether the North Karelian fig ures differ statistically (t test $*p<0.05$ $**p<0.01$ $**p<0.001$) from the respective reference figures either for the baseline situation or for the observed change The tables have a break-down into urban and rural groups because the place of residence is in many ways important in terms of the risk factors and their changes in the com munity

In the results the following indicators are used for pre senting the risk factor and related changes *Current smoker* subject has been a regular smoker previously and has smoked during the preceding month *Serum cholesterol* casual fasting serum cholesterol value (high s-cholesterol ≥270 mg/100 ml) *Consumption of low fat milk or no milk* subjects who reported that they consume non fat or low fat (2 5%) milk or no milk at all *Consumption of butter on bread* subjects who reported that they use mainly butter on their bread *Amount of fat used on bread* the amount indicated by the subject at the personal interview with the aid of model pieces of bread *Systolic BP* casual systolic BP sitting position (high systolic BP ≥160 mmHg) *Diastolic BP* casual diastolic BP using the 5th phase ("high diastolic blood pressure ≥95 mmHg) *Antihypertensive treatment* use of any antihypertensive drug during the preceding week

In the second part we distinguish among the high risk people at the baseline the groups with clear risk factor reductions during the follow up This is based on discri minant analyses between these groups and the groups with no satisfactory reductions The variables used and their classification were as follows *Sex* male female *Age* year of birth *County* North Karelia reference county *Place of residence* urban rural *Education* total years of school *Income* total annual income of the family *Main occupation* farming or forestry other *Years of smoking* total years of regular smoking *Amount of smoking* total number of cigarettes cigars or pipes smoked per day (1972) *Inhalation* smoke inhaled always often seldom never (1972) *Opinion about own smoking* considers oneself to smoke much too much somewhat too much modestly (1972) *Serum cholesterol* mg/100 ml (1972) *Cholesterol measurement* serum cholesterol measured during the 2 years preceding the 1975 survey others *Diagnosed hypertension* hypertension diagnosed during the project period (1972–75) earlier others *BP measurement* BP measured during 2 years preceding the 1975 survey others *Angina pectoris* angina pectoris di agnosed during the project period (1972–75) earlier others

RESULTS

The proportion of current smokers (smoked during the preceding month) decreased among the North Karelian male cohort from 47 to 36% and from 46 to 40% among the cohort from the reference area The changes occurred in both rural and urban groups (Table III) The percentage of heavy smokers (≥25 cigarettes) changed among the North Karelia males from 11 to 9 and among the reference group from 9 to 8 In the cross tabulation it turned out that 3 and 6% of all male subjects had started and 14 and 11% had stopped smoking since 1972 in the North Karelian and the reference group respectively Among the female cohorts the percentage of smok ers decreased in North Karelia from 10 to 8 and increased in the reference group from 7 to 10 The change occurred in both the urban and the rural groups although smoking was more prevalent in towns than in the countryside (Table III) Among current smokers the average number of cigarettes smoked changed among males from 20 to 18 and among females from 9 to 8 in the North Karelia cohort and from 18 to 16 among males and from 9 to 7 among females in the reference cohort

Table IV shows the percentages of subjects with serum cholesterol values of 270 mg/100 ml or more in the two cohorts in 1972 and 1975 Both males and females had a reduction in North Karelia and an increase in the reference county the trends in the urban cohorts were however slightly the opposite

Table IV *Percentages of subjects with high serum cholesterol (≥270 mg/100 ml) among the cohorts in 1972 and 1975*

	North Karelia			Reference county		
	Urban	Rural	Total	Urban	Rural	Total
Males						
1972	35	48	45	35	47	42
1975	36	45	43	33	51	44
Females						
1972	24	48	43	31	47	39
1975	31	42	40	30	46	40

Table V *Percentages of subjects reporting con sumption of low fat milk or no milk among the cohorts in 1972 and 1975*

	North Karelia			Reference county		
	Urban	Rural	Total	Urban	Rural	Total
Males						
1972	53 **	29	33	34	23	28
1975	89	53	60	75	39	54
Females						
1972	61*	41	45	47	37	41
1975	93	63	70	74	53	63

In both areas the figures were considerably lower in towns than in the countryside Although the dif ferences in changes were small they were consist ent at all cholesterol levels Thus among males the percentages of subjects with very high serum cholesterol (≥310 mg/100 ml) changed in North Karelia from 20 to 18 and in the reference group from 18 to 19 and the percentages of those with low serum cholesterol (<230 mg/100 ml) changed from 23 to 25 and from 25 to 21 respectively The same is shown by the mean cholesterol values among the male cohorts they changed from 267 to 264 in North Karelia and from 264 to 266 mg/100 ml in the refer ence group

As the programme aims at lowering the serum cholesterol level of the population the quality of y food stuffs esp dairy products is of central A great proportion of the fat consumed inates from milk The main choices are whole ilk or normal milk which are high in fat and low fat milk or butter milk which are low in fat The programme aims at a change from the fatty products to the low fat products or other non fat drinks The largest amount of fat is consumed with bread The programme aims at reducing this amount and at changing its quality to increase the P/S ratio of the fat The following results refer to these key indicators of the dietary changes

In 1972 the percentages of people who consumed low fat products or no milk at all were naturally higher in urban areas During the follow up this percentage increased among all the cohorts and practically as much among the reference as the North Karelian cohorts (Table V) At the same time there were no major changes in the amount of milk consumed

In 1972 the use of butter on bread was more common in the countryside than in the urban areas During the follow up the percentages of butter users decreased in both the urban and the rural cohorts and in both counties The decrease in North Karelia was more marked but not significantly (Table VI) The non users consumed mainly mar garine

The amount of fat used on bread was estimated at the personal interview by using model pieces of bread Table VI shows that females in general re ported using considerably less fat on their bread than males In 1972 a considerably higher per centage of the subjects in North Karelia than in the reference county reported using a minimum of 10 g fat on their bread In 1975 a great reduction had taken place among the North Karelian cohort while there had been an increase in the reference cohort Again the changes took place at all levels the percentages of males using 2 5 g or less fat on their bread increased from 5 to 13 among the North Karelian cohort and fell from 11 to 8 among the reference cohort

The percentages of subjects with elevated casual systolic BP (≥160 mmHg) decreased in both groups and for both sexes The decrease was consistently more marked among the North Karelian cohorts than among the respective reference cohorts al though not all the differences were statistically sig nificant (Table VII)

Table VI *Percentages of subjects using butter and those using at least 10 g fat on their bread among the cohorts in 1972 and 1975*

	North Karelia			Reference county		
	Urban	Rural	Total	Urban	Rural	Total
Butter users						
Males						
1972	66	91	86	76	92	85
1975	51	79	73	65	81	75
Females						
1972	67	84**	81	80	92	86
1975	43	67	62	64	73	69
Users of ≥100 g fat						
Males						
1972	46	64 *	60	26	46	38
1975	20 **	36	33*	48	47	48
Females						
1972	26**	33***	31***	12	19	16
1975	8 **	11**	11* *	18	23	21

Table VII *Percentages of subjects with elevated BPs among the cohorts in 1972 and 1975*

	North Karelia			Reference county		
	Urban	Rural	Total	Urban	Rural	Total
Systolic BP 160 mmHg						
Males						
1972	9 *	24	21	19	22	20
1975	2*	17	14	22	18	19
Females						
1972	22	31	29	16	33	25
1975	6 *	19	16*	17	24	21
Diastolic BP 95 mmHg						
Males						
1972	22***	33	31*	40	39	39
1975	18	43*	37**	29	34	32
Females						
1972	25	38	35	24	35	30
1975	15	53	29	22	30	26

Of the male subjects with high systolic BP (160 mmHg) in 1972 16% in North Karelia and 49% in the reference group had a pressure below 160 mmHg in 1975 On the other hand 7 and 11% respectively of those males with systolic BP below 160 mmHg in 1972 had a pressure above 160 mmHg in 1975

The respective figures for diatolic BP resemble those for systolic for the urban cohorts For the North Karelian rural male cohort an increase was found from 33 to 43% (Table VII) Of the rural males who in 1972 had a diastolic BP of 95 mmHg or more 37% in the North Karelian cohort and 44% the reference cohort had a pressure below 95 mmHg in 1975 On the other hand 32 and 20% respectively of those with diastolic pressure less than 95 mmHg in 1972 had 95 mmHg or more in 1975 In towns 55% of the hypertensives in North Karelia and 48% in the reference county had their diastolic pressure normalized in 1975 and 11% and 13% respectively developed diastolic hypertension

After the survey in 1972 the increase in antihypertensive drug treatment was rather similar among the cohorts Already in 1972 females had been on drug treatment more frequently than males the relative increase in frequency of drug treatment was however higher among males (Table VIII)

In order to further analyze the factors associated with a favourable risk factor at the individual level

Table VIII *Percentages of subjects on antihypertensive drug treatment among the cohorts in 1972 and 1975*

	North Karelia			Reference county		
	Urban	Rural	Total	Urban	Rural	Total
Males						
1972	3	3	3	5	5	4
1975	10	12	12	10	12	11
Females						
1972	5	9	8	6	9	7
1975	10	21	18	13	18	16

discrimination analyses were made for each of the risk factors separately

Subjects smoking regularly in 1972 were classified into two groups according to the follow up result in 1975 1) Stopped smoking (not smoking during the preceding month $N=77$) 2) Continued smoking (smoking during the preceding month $N=359$) Table IX gives the results from the discrimination analysis of the total study population concerning which factors and how strongly were simultaneously associated with stopping or continuing smoking on the individual level

Stopping smoking was found to be associated especially with less years of smoking younger age and diagnosed hypertension In addition it was associated with opinion about own smoking (too much) higher income male sex higher education and occupation (other than farming and forestry) In

Table IX *Results of discrimination analysis between the groups that stopped or continued smoking out of the current smokers in 1972*

	Coefficient of the normal variable	Correlation between the function and the variable
Years of smoking	91	50
Age	59	19
Diagnosed hypertension	53	47
Opinion about own smoking	35	31
Income	35	45
Sex	28	02
Education	26	42
Occupation	14	34
County	12	

the combination of these variables also county (North Karelia) added to the frequency of stopping smoking Only a slight association was found with no angina pectoris greater amount of smoking and urban place of living The developed model classified correctly 82% of the subjects in this material

For analysing cholesterol reductions the subjects with serum cholesterol levels of 279 mg/100 ml or higher in 1972 were classifed into two groups according to the follow up result in 1975 1) Cholesterol reduced (by 30 mg/100 ml or more $N=188$) 2) Cholesterol not reduced (unchanged or reduced by no more than 10 mg/100 ml $N=325$) The arbitrary limit of 30 mg/100 ml makes a real cholesterol reduction in group 1 likely because the changes in observed values could hardly be due to intraindividual variations or in accuracy of the measurement At the same time group 2 can be considered to have no cholesterol reduction because even an observed reduction of no more than 10 mg/100 ml on the individual level is either due to the above mentioned facts or unimportant The results of the discrimination analysis are shown in Table X

A high serum cholesterol level at the examination in 1972 was strongly associated with a clear reduction at the follow up This result includes the consequences of the cholesterol lowering changes and the phenomenon regression towards the mean Also higher education main occupation (other than farming and forestry) and place of residence (rural) are among the chief features of the group with cholesterol reduction in the analysis Living in the county of North Karelia was favourable for cholesterol reduction when considering the variables simultaneously In addition younger age and

Table X *Results of the discrimination analysis between the groups with and without cholesterol reduction out of the subjects with serum cholesterol values of ≥270 mg/100 ml in 1972*

	Coefficient of the normal variable	Correlation between the function and the variable
Serum cholesterol in 1972	90	85
Education	33	29
Main occupation	31	28
Place of residence	25	11
County	12	19
Age	10	16

Table XI *Results of the discrimination analysis between the groups with and without satisfactory change in BP out of the subjects with a BP of at least 175 and/or 110 mmHg in 1972*

	Coefficient of the normal variable	Correlation between the function and the variable
Sex	68	71
Age	60	34
County	41	45
Angina pectoris	38	39
Education	19	01
Place of residence	18	00
Main occupation	11	07
BP measurement	11	08

male sex were to some extent associated with cholesterol reduction at the follow up while angina pectoris cholesterol measurement income and diagnosed hypertension had very little association The developed model classified correctly 70% of the subjects in this material

For analysing reduction of high BP the subjects with a BP of at least 175 mmHg systolic and/or 110 mmHg diastolic in 1972 were classified into two groups according to the follow up value in 1975 1) BP reduced to normal (less than 160 systolic and 95 mmHg diastolic at the follow up $N=52$) 2) BP not satisfactorily reduced (at least 170 systolic and/or 105 mmHg diastolic at the follow up $N=115$) The limits were arbitrarily chosen but for intervention purposes group 1 can be considered to have a satisfactory reduction of high BP and group 2 not The results of the discrimination analysis are given in Table XI

A proper reduction of BP was associated to the greatest extent with female sex and younger age Living in North Karelia was clearly associated with having more often a satisfactory reduction of BP In addition no angina pectoris and to a lesser degree lower educational level and urban place of residence were associated with a proper reduction of BP Of less importance were occupation frequency of BP measurements and income The developed model classified correctly 68% of the subjects in this series

DISCUSSION

The present study is part of the research carried out within the North Karelia project to evaluate a

community programme for control of CVD in a comprehensive way This is a longitudinal follow up study of a subsample *originally* examined at the beginning of the programme in the intervention and reference areas It does not form part of the main evaluation of the programme i e the assessment of the risk factor changes induced by the programme *in the community* the reason being that at the out set the examination per se causes potential changes in the risk factor level of the sample These changes are not representative of the whole population in the community

It has been shown that screening without any other systematic intervention can cause consider able changes in risk factors (1) Thus the sample examined originally is no longer representative of the community as a whole and the assessment of the effect of the programme in the whole community has to be based on surveys of independent random samples This is why in the large five year terminal survey in the spring of 1977 an independent ran dom sample is used in North Karelia and a refer ence county Also in the inbuilt continuous evalua tion development in North Karelia in terms of health behaviour of the population is followed with smaller independent samples (2)

The assumption that a sample examined at the baseline and another independent sample can differ e g at a three year follow up is illustrated by com paring smoking habits of North Karelian males from this study and of an independent representative random sample from another postal survey which was carried out in the spring of 1975 in the com munity (participation rate 86% $N=2300$) The percentage of smokers in North Karelia was 51 in the baseline survey (1972) and in the 20% subsam ple that was studied three years later it was 47 in 1972 and 36 in 1975 At the survey of the independ ent sample in 1975 the percentage was 44 Observ ing the same people the situation in the communi ty in 1975 might thus look better than it really is possibly due to the impact of the examination of the sample in 1972 The figure from the independent follow up survey is more likely to represent the true situation in the whole community Also for the ref erence area the method of this study is not likely to give a true picture The slight difference in the 20% subsample compared with the whole sample in 1972 might be due to sampling variation and the bias from the drop-out during the three years because the group includes only subjects who could be fol lowed for the three year period Because of these aspects this study forms only an additional form of evaluation of the community programme It can however be used to characterize the risk factor changes that have taken place during the three years on the individual level

The changes in smoking habits seem to indi cate a more favourable process among the North Karelian cohort than in the reference area Among males a clear reduction is observed and among the females the general tendency to increase may be prevented in North Karelia The multivari able analysis gives information about the determi nants of change in this habit Some of the findings are quite expected thus less years of smoking sub jective worry about the habit and diagnosed hyper tension promote the cessation of smoking On the other hand some of the findings within this group are in contrast to or at least not seen when following the changes among the whole of the North Karelian population this is the case e g with age and edu cation In North Karelia a greater reduction is seen among the older and among the less educated popu lations The differences can be due to the above mentioned different types of follow up and the pos sible impact of the reference area in this study the process there may be different In any case the multivariable analyses confirm the more favourable development in North Karelia

The serum cholesterol changes observed in this study are small However in the North Karelian countryside a reduction is seen in the cohort com pared with the reference cohort Some of the main indicators of the diet show favourable changes in the total series The North Karelian cohort shows however a greater change in many aspects This is the case especially with the amount of fat used on bread the North Karelian cohort showing a sub stantial reduction compared with some increase among the reference group Of course it cannot be said whether the answers represent the true situa tion or whether the North Karelians tend to report a better situation than is actually the case Also the type of fat consumed should be taken into consider ation

The multivariable analysis of the people with cholesterol reduction confirms also the more favourable change in North Karelia compared with the reference area in connection several other factors In addition higher education other occu pation than farming and fore a[illegible] [illegible]

residence seem to predict cholesterol reduction On the other hand a number of clinical factors like diagnosed angina pectoris hypertension or further cholesterol measurements are surprisingly enough of practically no importance

The method of following the same people is especially inappropriate for evaluating a community hypertension programme because at the baseline survey every subject with elevated BP is directed to control measurements and possible treatment in both areas This is reflected by the similar increase in the proportion of treated subjects However the proportion of high systolic BPs decreased more among the North Karelian cohorts than among the reference cohorts Surprisingly for high diastolic BP the opposite is found among the males It cannot be said whether this represents a true situation or could be due to some differences in the measurement techniques The latter might be the case because a major explanation for the result lies in the unexpected number of subjects in North Karelia with diastolic pressure over 95 mmHg in 1975 among those who had a value below 95 in 1972 When considering the somewhat unexpected changes in BP on the individual level from 1972 to 1975 it should be born in mind that we are dealing with casual BP values

The multivariable analysis shows that a satisfactory reduction of BP was experienced especially by younger subjects females and in North ... It also seems to refer to subjects with no ...ina pectoris and with less education The findings could be at least partly due to the normalized group having less severe hypertension at the baseline

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Premonitory Symptoms and Stress Factors Preceding Sudden Death from Ischaemic Heart Disease

Viljo Rissanen, Matti Romo and Pentti Siltanen

From the University of Kuopio, Kuopio, and the Research Department of the Finnish Heart Association, Helsinki, Finland

ABSTRACT. Premonitory symptoms as well as acute and long-standing stress preceding death were studied in 118 cases of prehospital sudden death, 62% of whom had shown premonitory symptoms. Prodromes were found in 94% of those whose fatal attack lasted longer than 2 hours. Premonitory symptoms seemed often to be unspecific in nature in cases of sudden death compared with those experienced by survivors of acute myocardial infarction. The occurrence of prodromes correlated with normal heart weight but not with the severity of the coronary artery disease or the presence of coronary thrombosis. Heavy smoking and a definite myocardial infarction revealed at autopsy were more frequent in those who had prodromes classifiable as unstable angina than in those with unspecific symptoms or without prodromes. The significance of acute and long-standing stress was most evident in the fatality of subjects with no long history of clinical disease. Although stress factors seemed to modify the course of the attack, a basic factor in the fatality was the coronary artery disease of critical severity. Stress factors did not play a major role in the precipitation of premonitory symptoms. Stress in patients with triple vessel disease in the coronaries was, however, more frequently (88%) conductive to prodromes than in those with double or single vessel disease (50%).

Studies on patients dying either suddenly or non-suddenly from an acute myocardial infarction (AMI) (7, 12, 13, 14, 16) have shown that in many cases a symptomatic phase antedated the acute attack. The experience gained at coronary care units indicates that a high proportion of early deaths are arrhythmic in origin. In the hope of preventing the development of dangerous arrhythmias, great attention has been paid to the identification of patients liable to an acute attack in the near future. Epidemiologic prospective studies have shown that it is impossible to predict from the profile of generally accepted risk factors whether death from a coronary disease will be sudden or not (3). The recognition of premonitory symptoms could be valuable in identifying patients who are most vulnerable to an acute episode of ischaemic heart disease (IHD). Identification and elimination of precipitating factors conductive to sudden death might also contribute to prevention. Thus, emotional stress, type A behaviour pattern and life change events have been added to the list of risk factors of IHD (4). The association of these factors with the suddenness of death as well as with premonitory symptoms preceding death has not been fully documented.

The purpose of this study was to analyse the occurrence of premonitory symptoms and stress-producing events before a sudden fatal attack of IHD and to analyse the correlations of prodromes and stress factors to the postmortem findings of the myocardium and the coronary arteries in a series collected by the Ischaemic Heart Disease Register of Helsinki.

PATIENTS AND METHODS

The original series consisted of 151 cases of sudden prehospital death from IHD in Helsinki during a period of 12 months. In all cases the fatal attack was witnessed, death occurred within 24 hours from the onset of the attack, and special methodology was applied to the study of the heart at autopsy (11). The present study was based on a series of 118 subjects, 94 men aged 31–83 years (mean 56.6) and 24 women aged 44–78 years (mean 58.8), in whom information on circumstances during 28 days preceding death was obtained from relatives or friends. Information on the fatal attack, previous diseases, premonitory symptoms and stress-producing events preceding death was obtained from hospital records and from relatives or friends. The principles of the data collection carried out by the

Abbreviations: MI, myocardial infarction; AMI, acute MI; IHD, ischaemic heart disease.

Address for reprints: Dr V. Rissanen, University Central Hospital, SF 70210 Kuopio 21, Finland.

Table I *Prevalence of premonitory symptoms*

	No of cases	%
Changed angina pectoris	18	15
Recent angina pectoris	7	6
Dyspnoea on exertion	18	15
Dysrhythmia	6	5
Discomfort in the chest	28	24
Heaviness of the arms	4	3
Unusual fatigue	38	32
Malaise sense of raised temperature etc	6	5
Nausea vomiting	5*	4
Sweating	3	3
Nervousness depression	3	3
Cardiac symptoms (changed or recent angina pectoris dyspnoea dysrhythmia)	39	33
Possible cardiac symptoms (discomfort in the chest heaviness of the arms)	13	11
Other symptoms	21	18
Total	73	62

* One had used digitalis

Ischaemic Heart Disease Register of Helsinki have been previously described in detail (12)

Premonitory symptoms during 28 days before death were recorded according to the principles outlined by the WHO (17) In addition data on the circumstances connected with the fatal illness were collected in all cases According to their relatives or friends 30 subjects had experienced stress due to troubles or haste at work troubles in the family or elsewhere distress etc during 28 days before death (long standing stress) Acute stress i e emotional excitement distress exceptional physical exercise or effort in the 12 hours before the attack was recorded in 23 cases The relatives of 65 subjects did not introduce any facts which could indicate a stress preceding death

A history of previous angina pectoris myocardial infarction (MI) or dyspnoea on exertion was recorded according to the definitions of the WHO Medication was recorded paying particular attention to the use of digitalis or diuretics For smoking habits the patients were divided into four groups 1) heavy smokers (25 or more cigarettes a day) 2) smokers 3) ex smokers (given up smoking at least 3 months before death) 4) non smokers

Autopsy data

Medico-legal autopsies were carried out at the Department of Forensic Medicine University of Helsinki The methodological principles outlined by the WHO (15 18) were applied in the postmortem examination of the hearts A detailed description of the methods has been given in a previous paper (11) The degree of coronary stenosis was estimated on the basis of a postmortem coronary angiography and examination of the longitudinally opened coronary arteries A 50% stenosis was regarded as a significant involvement of a coronary artery

The two stages of a recent MI were defined on the basis of macroscopic and microscopic examinations of the myocardium as follows *Initial MI* macroscopic discolouration pallor or softening and/or microscopic changes without leucocytic infiltration *Definite MI* macroscopic necrosis softening or discolouration of the myocardium and infiltration of polymorphonuclear leucocytes in addition to other changes in microscopy A scar 0 5 cm or more in diameter was regarded as an old healed infarction The presence of a fresh coronary thrombosis was estimated visually from angiograms and the longitudinally opened coronary arteries

RESULTS

Prevalence of premonitory symptoms

Premonitory symptoms had occurred in 62% of the patients More than half of them (1/3 of all cases) had had symptoms probably of cardiac origin i e angina pectoris of recent onset changing pattern of previous anginal pain dyspnoea or dysrhythmias (Table I) Unusual fatigue was one of the major symptoms in every second patient with prodromes preceding the fatal attack As the only symptom it was present in 8% of the cases Another rather unspecific symptom discomfort in the chest was also fairly frequent

The fatal attack in relation to premonitory symptoms

The great majority of the patients died very suddenly i e instantaneously or within 2 hours from the onset of the attack Of the 17 subjects who died

Table II *Suddenness of the fatal attack and stage of recent MI verified at autopsy in 73 patients with and 45 without premonitory symptoms*

	Pats with premonitory symptoms		Pats without premonitory symptoms	
	No of cases	%	No of cases	%
Fatal attack				
Instantaneous	35	48	22	49
Less than 2 h	22	30	22	49
Longer than 2 h	16	22	1	2
MI				
Definite	24	33	9	20
Initial	32	44	24	53
No	17	23	12	27

Table III *Prevalence of coronary thrombosis in relation to presence or absence of premonitory symptoms and stage of recent MI found at autopsy*

	Pats with premonitory symptoms			Pats without premonitory symptoms		
	No of cases	Pats with thrombus		No of cases	Pats with thrombus	
		n	%		*n*	%
Definite MI	24	19	79	9	6	67
Initial MI	32	16	50	24	14	58
No MI	17	4	24	12	3	25
Total	73	39	53	45	23	51

after an attack lasting longer than 2 hours 16 had a history of premonitory symptoms (Table II) A definite MI verified at autopsy was slightly but not significantly more common in patients with than without prodromes Of the patients with a definite AMI revealed at autopsy 73% had shown premonitory symptoms The corresponding figure for patients with no detectable AMI was 59% Coronary thrombosis was related to the presence and stage of an AMI but not to the history of premonitory symptoms (Table III)

Correlations between premonitory symptoms and other parameters

No significant correlation was found between the occurrence of premonitory symptoms and the age or sex of the patients or history of previous IHD treatment with digitalis or diuretics smoking habits and autopsy findings of the coronary arteries Prodromes were less frequent in patients with marked cardiac hypertrophy than in those with no marked enlargement of the heart ($p<0.05$) (Table IV)

Table IV *Occurrence of premonitory symptoms in different subgroups of sudden deaths*

	Total no of pats	Pats with premonitory symptoms	
		No of cases	%
Males	94	57	61
Females	24	16	67
60 y or older	45	25	56
Younger than 60 y	73	48	66
History of previous IHD	86	51	59
No history of previous IHD	32	22	69
Heavy smokers	32	23	72
Smokers and ex smokers	61	35	57
Non smokers	25	15	60
On digitalis or diuretics	43	26	60
Not on digitalis or diuretics	75	47	63
Marked cardiac hypertrophy*	62	33	53
No marked cardiac hypertrophy	56	40	71
Old MI at autopsy	77	45	58
No old MI	41	28	68
Triple vessel disease in coronaries	66	40	61
Double vessel disease	39	25	64
Single vessel disease or no coronary stenosis	13	8	62

* Heart weight≥500 g in men ≥450 g in women

Table V *Prevalence of previous smoking habits cardiac disease and stress factors in patients with and without premonitory symptoms*

	Pats with premonitory symptoms									
	Angina pectoris of recent onset (*n*=7)		Other cardiac symptoms (*n*=32)		Possible cardiac symptoms (*n*=13)		Other symptoms (*n*=21)		Pats without prodromes (*n*=45)	
	No of cases	%	No of cases	%	No of cases	%	No of cases	%	No of cases	%
Smokers total	*5*	*71*	*21*	*65*	10	77	10	48	23	51
Heavy smokers	4	57	11	34	6	46	2	10	9	20
Previous MI	0	0	9	32	2	15	6	29	13	29
Previous cardiac symptoms	3*	43	31	97	9	69	10	48	36	80
Therapy with digitalis or diuretics	1	14	16	50	3	23	6	29	17	38
Acute stress	*0*	*0*	*6*	*19*	*5*	*38*	*4*	*19*	*8*	*18*
Long standing stress	6	86	5	16	4	31	6	29	9	20

* Dyspnoea on exertion

Heavy smoking was more common (40%) in patients with premonitory symptoms clearly or possibly of cardiac origin than in those with unspecific symptoms or no prodromes (20%) ($p<0.01$) (Table V) No difference in the occurrence of various types of premonitory symptoms was found between patients *who had used digitalis and/or diuretics and* those who had not been on this kind of medication before they died A definite AMI verified at autopsy was more common in subjects with premonitory ptoms clearly or possibly of cardiac origin %) than in those with unspecific premonitory toms or no prodromes (20%) ($p<0.05$) The tter two groups had very similar results for most of the comparisons (Tables V and VI) No differences were found in the severity of the coronary artery involvement between patients with different types of premonitory symptoms or between them and patients with no prodromes

Stress factors preceding death

Twenty five per cent of the patients had a history of long standing stress and 19% of some acute stress preceding death Sudden fright at the onset of the fatal attack was known in one subject with a ruptured MI revealed at autopsy None of the patients with acute stress had a fatal attack lasting longer than 2 hours (Table VII) and sudden death with no detectable recent MI was most frequent among them On the other hand long standing stress was often followed by a fatal attack terminating within 2–24 hours from the onset A definite AMI was most often found to succeed long standing stress In this respect these patients differed significantly from those with acute stress ($p<0.05$) No differences in the severity of the coronary artery disease were found between patients with acute or *long standing stress or between them and those* with no known preceding stress

Of the 7 patients with a history of angina pectoris of recent onset 6 had also a history of long standing stress recorded within 4 weeks before death (Table V) The severity of the coronary artery disease in these 7 patients was comparable with that of the other patients in the series (Table VI)

Of the 10 patients with neither a history of IHD nor prodromes 9 had suffered from long standing and/or acute stress before death Thus all but one a *78 year-old woman of the 118 subjects had had* either a history of previous IHD or premonitory symptoms or stress preceding death

Premonitory symptoms and stress factors

The prevalence of premonitory symptoms tended to be higher (70%) in patients with long standing stress than in those with no known stress (55%) (p=n s) All patients in whom long standing stress was followed by a late death (2–24 hours Table VII) had experienced premonitory symptoms 65% of the patients with acute stress *had suffered from* prodromes On the other hand only 29% of all premonitory symptoms were associated with

Table VI *Prevalence of autopsy findings of the heart in patients with and without premonitory symptoms*

	Pats with premonitory symptoms									
	Angina pectoris of recent onset ($n=7$)		Other cardiac symptoms ($n=32$)		Possible cardiac symptoms ($n=13$)		Other symptoms ($n=21$)		Pats without prodromes ($n=45$)	
	No of cases	%	No of cases	%	No of cases	%	No of cases	%	No of cases	%
Definite recent MI	4	57	12	37 5	4	30	4	19	9	20
Initial recent MI	3	43	12	37 5	8	62	9	43	24	53
No recent MI	0	0	8	25	1	8	8	38	12	27
Old MI	3	43	22	69	10	77	10	48	32	71
Coronary occlusion	5	71	22	69	11	85	15	71	39	87
Triple vessel disease	5	71	17	53	7	54	11	52	27	60
Double vessel disease	1	14	13	41	5	38	6	29	14	31
Single vessel disease or no stenosis	1	14	2	6	1	8	4	19	4	9
Coronary thrombosis	4	57	19	59	7	54	10	48	24	53
Marked cardiac hypertrophy	4	57	14	44	3	23	10	48	29	64

Heart weight≥500 g in men ≥450 g in women

known long standing stress factors during 28 days preceding death A preceding long standing stress was recorded only slightly less frequently (20%) in patients with no prodromes (Table V) Among subjects who had a history of long standing stress premonitory symptoms occurred more frequently in those with triple vessel disease (88%) than in those with double or single vessel disease (50%) ($p<0\ 05$) There was no difference in the quality of the symptoms

DISCUSSION

Premonitory symptoms were frequent (62%) in the present series An analysis of all acute episodes of IHD in Helsinki within a year showed that the frequency was highest in the survivors of AMI (67%) followed by the non sudden fatal cases (64%) and lowest among cases of sudden death (55%) (12) The frequency of reported angina pectoris was definitely higher in the survivors (35%) than in the cases of non sudden (22%) and sudden deaths

Table VII *Suddenness of the fatal attack stage of recent MI verified at autopsy and severity of coronary artery disease in patients without and with acute or long standing stress preceding death*

	Pats with acute stress ($n=23$)		Pats with long standing stress ($n=30$)		Pats without stress ($n=65$)	
	No of cases	%	No of cases	%	No of cases	%
Fatal attack						
Instantaneous	14	61	11	37	32	49
Less than 2 h	9	39	9	30	26	40
Longer than 2 h	0	0	10	33	7	11
MI						
Definite	2	9	12	40	19	29
Initial	12	52	14	47	30	46
No	9	39	4	13	16	25
Coronary artery disease						
Triple vessel disease	14	61	16	53	36	55
Double vessel disease	7	30	10	33	22	34
Single vessel disease or no stenosis	2	9	4	13	7	11

(16%) The latter figure is very similar to the corresponding prevalence in the present series With regard to preventive measures it is unfavourable that the prodromes in patients who died suddenly were specific of IHD less often than those experienced by the patients surviving an AMI

A definite AMI could be verified at autopsy more often in patients with premonitory symptoms clearly or possibly of cardiac origin than in those with unspecific symptoms or no prodromes The occurrence of premonitory symptoms has obviously had some influence on the composition of the present series Sixteen out of the 17 subjects whose fatal attack lasted longer than 2 hours had a history of premonitory symptoms Since nearly all cases of prehospital deaths in Helsinki are medico-legally autopsied we must assume that patients who had no prodromes but did have an attack lasting longer than 2 hours were alive when they arrived at the hospital If they died in the hospital they will have been autopsied elsewhere than the present cases On the other hand in certain cases the premonitory symptoms had evidently interfered with the interpretation of the nature of the acute attack and caused delay in summoning aid Among these patients there might be some in whom the premonitory symptoms were manifestations of an ongoing infarction i e they did not show abrupt severe symptoms indicating the onset of an infarction Particularly in elderly people unspecific symptoms f an infarction may be interpreted as a banal condi like influenza

it is unfortunate that the most common pre nitory symptoms undue fatigue and discomfort in the chest are unspecific of a cardiac disease In the present series a changing pattern of previous angina pectoris or angina of recent onset was found in 21% of the cases which is in accordance with the results of Simon and Alonzo (13) Dyspnoea on exertion and discomfort in the chest as well as heaviness of the arms should probably be added to these more typical features of unstable angina If these less specific symptoms are included the prevalence of unstable angina rises to 44% Unspecific symptoms do not however help a clinician to identify a patient liable to sudden death or to make decisions concerning therapy A Finnish population study (10) showed that 7–12% of men and women aged 50–59 years had a history of typical angina pectoris 14% of men and 11% of women had chest pain symptoms associated with effort but not typical for angina pectoris and one third of both sexes reported chest pains not associated with effort The high prevalence of chest pain symptoms or discomfort in the chest in the middle aged population and the frequent unspecificity of the symptoms make interpretation by practitioners difficult When a patient has a definite diagnosis of IHD and the symptoms show a changing tendency the problem is simpler

Unstable angina pectoris as a preinfarction angina is an important syndrome between stable angina pectoris and acute MI (1) Patients certainly at high risk level are those whose pain persists in bed rest Hospitalization bed rest and drug treatment are usually employed when unstable angina manifests itself as angina on effort with a changing pattern or as angina at rest in a patient with previous angina on effort On the other hand at most centres patients with angina on effort of recent onset are considered less at risk and are treated in ambulatory care by limiting their activities (1) The justification of this practice is supported by a comparison of clinical experience and the present results Out of the 167 patients with unstable angina examined in a project of general practitioners in Edinburgh 88 (53%) had angina on effort of recent onset (5) Only 3 patients in the total series died suddenly within three months In general only in a small proportion of all sudden deaths is angina of recent onset involved (12 13) In the present series the prevalence was 6% Patients in whom stress producing events are associated with a recent onset of angina pectoris seem to be in greatest danger of dying

The pathophysiologic background of premonitory symptoms has not been fully documented As in the present study they are not related to a particular patho-anatomic pattern of the coronary artery disease (6) nor are they a manifestation of a thrombotic tendency in the coronaries (Table III) Premonitory symptoms frequently seem to involve primary cardiac events of unknown pathophysiologic mechanisms (1) Extracardiac precipitating factors e g stress factors may result in prodromes by increasing the oxygen demand of the myocardium (2) In the present series stress factors did not seem to be a major factor in the precipitation of premonitory symptoms long standing stress was known in less than one third of the subjects who had experienced prodromes On the other hand the prevalence of premonitory symptoms tended to be highest in persons with long standing stress It

should be noted that long standing stress in patients with triple vessel disease in the coronaries was more frequently (88%) conductive to prodromes than in those with double or single vessel disease (50%) The more severe the coronary artery dis ease the greater is the liability to myocardial ischaemia Premonitory symptoms may be reflec tions of the functional disturbances in the heart provoked by ischaemia Hence it is easy to under stand the sensitive occurrence of prodromes in as sociation with long standing stress particularly in patients who are most liable to ischaemia i e those with a most severe coronary artery disease

The probability of sudden death without preced ing premonitory symptoms seems to increase with the progress of the ischaemic disease to a stage with marked cardiac hypertrophy In the present series heavy smoking a well known risk factor of IHD was particularly related to the occurrence of prodromes classifiable as unstable angina The se verity of the coronary artery disease does not seem to correlate any better with the occurrence of pre monitory symptoms classifiable as unstable angina than with the unspecific symptoms Other studies have shown that the various categories of angina pectoris i e recent stable and unstable are not related to the severity of the coronary artery dis ease (6) Follow up studies have shown that the death rate in patients with unstable angina is rather low and probably not much different from that in patients with stable angina pectoris with a similar degree of coronary artery narrowing (8)

A careful and detailed study of all stress factors e g life change events during the antemortem year (9) was not possible in the present study The available information showed that the significance of the preceding stress producing events was most evident in the fatal attacks of patients with no long standing clinical disease i e those with angina of recent onset and those with neither a history of previous IHD nor prodromes Stress factors pre ceding the acute attack seemed to modify the course of the attack Acute stress was often as sociated with a very rapid fatal outcome and no detectable AMI at autopsy On the other hand long standing stress was frequently followed by a more prolonged attack with a definite AMI verified at autopsy In the latter cases death was mostly preceded by premonitory symptoms

Since the data were collected from the relatives or friends of the patients it is possible that a posi tive history of a preceding stress was mainly given by those who were liable to express their feelings On the other hand it is obvious that the inclusion of stress cases in the group of no stress should have weakened the result concerning the influence of the stress factors on the parameters studied

It is clear that both long standing and acute stress producing events may influence the symp tomatology and progress of IHD Stress may also precipitate acute attacks On the other hand even though the onset of an acute attack might be pre cipitated by stress factors fatal outcome seems to occur only if the involvement of the coronary arteries has reached a critical level of severity This is in accordance with the conclusions made in clini cal angiographic studies which have shown that the severity of obstructive coronary artery disease is a major factor determining the mortality in IHD pa tients (8)

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Coronary Arteriographic Findings in Patients with Previous Acute Myocardial Infarction

J Fischer Hansen

From Medical Department B Rigshospitalet Copenhagen Denmark

ABSTRACT In a consecutive series of 234 patients admitted for selective coronary arteriography, 49 had had definite acute myocardial infarction (AMI) three months to ten years previously More than 75 % stenosis in at least one of the major coronary arteries was found in 80 % of the patients Two and three vessel disease was demonstrated in 31 % of the pa tients, which differs significantly from the 75–80 % reported in autopsy studies in patients dying from AMI At ventriculography all 22 patients with pathological Q waves had dyssynergy of the left ventricle

Our knowledge of changes in the coronary arteries in patients with acute myocardial infarction (AMI) is mainly based on necropsy findings (7 20) Only a few investigations have covered the changes in the coronary arteries in patients surviving a trans mural AMI diagnosed by history clinical findings electrocardiography (ECG) and changes in serum enzymes (1 2 22) The changes demonstrated by coronary arteriography in patients surviving an AMI seem to be substantially less pronounced than those revealed at necropsy in patients who died from an AMI In recent years several cases with normal or nearly normal coronary arteries have been reported in patients with previous transmural AMI (3 4 15) or non penetrating AMI (5) Further more the prognosis in patients with coronary ar tery disease (CAD) depends on the number of ves sels involved (13)

These circumstances together with the improved possibilities for treating CAD by bypass surgery make it of major interest to examine the degree of coronary artery changes in patients with previous AMI This is the purpose of the present commu nication

PATIENTS AND METHODS

In the period Aug 1968–June 1975 technically satisfac tory selective coronary arteriographies were performed in 234 patients The investigations were carried out with Jud kins technique usually including ventriculography in right anterior oblique projection (10 14) The patients were examined because of chest pain AMI at an early age and/or heart failure and only for diagnostic reasons as coronary artery surgery was not carried out in that period Forty nine patients without congenital or valvular heart disease fulfilled the WHO criteria for definite AMI (19) Ventriculography could not be performed at the be ginning of the period and is not available for 15 of the patients

All patients were admitted to a local hospital or to Rigs hospitalet on account of the acute event It was possible retrospectively on the basis of the records from these admissions to obtain information about history and clini cal findings changes in ECG and serum enzymes i e lactic acid dehydrogenase and/or glutamic oxaloacetic transaminase The time which elapsed from the acute in cident to the performance of the arteriography varied from three months to ten years Twenty-one patients were in vestigated in the 1st eight in the 2nd six in the 3rd two in the 4th three in the 5th and nine in the 6th–10th year after the AMI Forty three patients were males mean age 47 years (range 28–65) and six females mean age 46 (range 32–51) Angina pectoris was found in 25 patients 13 had atypical angina

The assessment of a fasting resting 12 lead ECG has been made in accordance with the Minnesota code (21) Pathological Q waves fulfill the code point 1 1 1 2 or 1 3 but without left hypertrophy (point 3 1 or 3 3) and right bundle branch block (point 7 2)

The assessment of the degree of stenosis of a coronary artery has been made in the projection where the stenosis is most pronounced The picture has been projected onto a screen (Tagarno Denmark) and limits of the vessel proximal to the stenosis and corresponding to the stenosis have been drawn on paper and measured with a slide gauge CAD denotes more than 75 % stenosis in one of the major coronary arteries i e right coronary artery the cir cumflex or the anterior descending branch of the left coro nary artery None of the patients had left main CAD

Dyssynergy stands for dys hypo- or akinesis of area of the left ventricle at ventriculography in terior oblique projection (12

Abbreviations AMI=acute electrocardiogram CAD=cr

RESULTS

The criteria for the diagnosis of AMI and the findings at coronary arteriography in the 49 patients were as follows 1) 39 had typical history serum enzyme changes and serial changes in ECG with development of Q waves 34 of these had CAD 2) 4 had typical history and ECG with Q waves 3 of these had CAD 3) 5 had typical history and enzyme changes 1 of these had CAD 4) 1 patient with typical enzyme changes and ECG with Q waves had CAD Thus 39 (80%) of 49 patients had CAD Of these 39 patients 33 had occlusion 5 had more than 90% stenosis and 1 had more than 75% stenosis of at least one vessel Four patients had three-vessel 11 two-vessel and 24 one vessel disease Of the 10 patients without CAD 3 had 25-50% stenosis 1 had less than 25% stenosis in one vessel and 6 had normal coronary arteries Three or two-vessel disease was thus found in 31% of the patients Ventriculography was performed in 34 patients Dyssynergy was found in 27 patients of whom 22 had CAD Of the 7 patients without dyssynergy 5 had CAD Thus 32 (94%) of 34 patients had either dyssynergy and/or CAD At the time of the arteriography 32 of the 49 patients had pathological Q waves 26 (81%) of these 32 had CAD Ventriculography was performed in 22 of the patients with pathological Q waves and all had dyssynergy of the left ventricle

DISCUSSION

All 49 patients satisfy the criteria for definite AMI (19) At the time of arteriography pathological Q waves had disappeared in 24% of the patients who had had pathological Q waves at the time of diagnosis of AMI which fact does not exclude previous transmural AMI (6) Three or two-vessel disease was found in 31% of the patients a finding that is significantly different from recently reported figures 76 and 82% found at necropsy in patients who died from transmural AMI (7 20) Our findings are more in accordance with the fact that three and two-vessel disease was found in 45% of 38 patients (22) and in 47% of 68 patients (1) with AMI verified by ECG history and enzymes In this connection it is of interest to note that Fischer (personal communication) found at necropsy three or two-vessel disease in 27 (61%) of 44 patients with infarct fibrosis and previous clinical and ECG verified AMI The difference in degree of CAD in patients who survive or die after an AMI agrees with the fact that the survival rate in patients with three or two-vessel disease is significantly lower than in patients with one-vessel disease (13)

Coronary occlusion was found in 67% of our patients Begg et al (2) found coronary occlusion in 37% of their 51 patients examined a few days to six weeks after an AMI but all had at least 50% stenosis in one vessel At arteriography of patients with previous AMI normal vessels have been described in 7 9 (22) 6 4 (15) and 4 2% (4) and slight changes in 5 1% (15) In the present series 12% had normal coronary arteries and 8% had only slightly changed coronary vessels In a Danish necropsy material (8) of 137 old myocardial infarcts transmural as well as non penetrating 10 9% had only slight or no changes in the coronary arteries (Fischer personal communication) The relatively high proportion of patients in the present series without CAD may be due to patient selection with a high number of younger patients

Even though AMI in the majority of patients in all age groups is accompanied by CAD it appears that a significant number especially young patients have normal or almost normal vessels and a great number have no occlusion even though they have CAD The reason why no occlusion can be shown may be 1) dissolution of the thrombus and/or recanalization (4 9) and dilatation of the artery (8) 2) AMI is caused by coronary artery spasm which has been demonstrated in patients with normal vessels as well as in patients with CAD (17) 3) reduced myocardial blood flow because of non-relaxation of the myocardium during hypoxia (11 16) 4) misinterpretation of the arteriogram because the occlusion is at the very entrance of the artery (23) Pathological Q waves were accompanied by CAD in 81% and by dyssynergy of the left ventricle in all patients A similar high predictive accuracy of pathological Q waves for myocardial disease has been described earlier (18)

Based on the literature and the presented findings it seems reasonable to conclude 1) there is a significantly lower incidence of CAD in the patients who survive an AMI than in those who die from an AMI 2) a considerable number of patients with previous AMI have normal or nearly normal coronary arteries 3) pathological Q waves in patients with previous AMI are closely correlated to left ventricular dyssynergy

ACKNOWLEDGEMENTS

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Cerebral Attacks due to Excessive Vagal Tone in Heavily Trained Persons

A Clinical and Electrophysiologic Study

Verner Rasmussen Stig Haunso and Knud Skagen

From the Department of Cardiology Gentofte Hospital Copenhagen Denmark

ABSTRACT Cardiac syncopes appeared in four heavily trained male patients without a history of cerebral or heart disease Three were young athletes participating in competitive sport one had trained intensively for years after poliomyelitis complicated by paraplegia On admission all patients had sinus bradycardia, one had second degree atrioventricular (AV) block at rest and one had transient sinoatrial (SA) block His bundle studies demonstrated prolonged recovery time of the SA node (SAN) in two prolonged atrio-His interval in three and appearance of second degree AV block at abnormally low pacing rates in two Refractory periods of the AV node (AVN) determined in three tended to reach the upper limit of the normal range The dysfunction of SAN and AVN was temporarily abolished in all patients by 1 mg of atropine i v , and disappeared during exercise test which was done by the three young athletes The patient with paraplegia and one of the young athletes, who had second degree AV block at rest were given atropine 0 5 mg six times a day, and all three active sportsmen reduced training activity considerably After 6–12 months all patients were re-examined None had cerebral symptoms or other complaints They were in regular sinus rhythm and in excellent physical condition

Heavy physical training may cause anatomic and physiologic changes that are sometimes difficult to classify as either normal or pathological Cardiac enlargement ejection murmurs and various electrocardiographic (ECG) signs of increased vagal tone such as bradycardia prolonged P–Q intervals and ST T changes are common findings in top athletes and considered harmless (1) However in a few cases cardiac pacemakers and conductive tissue are so markedly depressed by the excessive vagal tone that cerebral blood flow may be impaired as a consequence of reduced cardiac output Four such cases are described in the present paper

SUBJECTS AND METHODS

Four heavily trained male patients entered the study None had a family history of cardiac or cerebral disease Three were young healthy athletes participating in competitive sport and physical training programmes One had a paraplegia after poliomyelitis in 1952 After his primary illness he was restored by intensive training for years so that he was enabled to walk on crutches and to drive a motor-car Further clinical data and details of training programmes are presented in Tables I and II respectively

A thorough clinical examination chest X ray repeated surface ECG bicycle exercise test and after informed consent a His bundle study were performed in all patients Patients 1 3 and 4 were advised to stop heavy training and patients 2 and 3 were given atropine 0 5 mg perorally six times a day All were re-examined and had a new exercise test 6–12 months after their first examinations

The His bundle studies were performed as described by Scherlag et al (12) with the introduction of a multipolar electrode catheter via the right femoral vein to the right side of the heart The catheter tip was positioned across the tricuspid valve in close proximity to the atrial septum Further catheters were introduced via a cubital or left femoral vein to the right atrium for pacing All intracavitary recordings were made on a six-channel ECG recorder (Elema Schonander Mingograph 81) The sensitivity was 200 μV/cm and the frequency band used was 50–700 Hz The equipment was carefully earthed at one point only to avoid earth loops A switch box connecting the intracavitary electrodes and the recording equipment had a built in current limiter (20 μA in case the patient was touching 220 V AC) All recordings were made at a paper speed of 100 mm/sec

RESULTS

ECG findings and results of exercise tests are listed in Table III Data on automatic activity and

Abbreviations AV=atrioventricular AVN=AV node AV nodal SA=sinoatrial SAN=SA node SA nodal ECG=electrocardiogram electrocardiographic HR=heart rate AH=atrio-His HV=His ventricular

Table I *Clinical data*

Pat. no	Age (y)	Symptoms	Clinical findings: Slow irregular pulse	Ejection murmur	Cardiac enlargement on X-ray
1	24	Postexercise syncope	+	−	+
2	37	Repeated Stokes-Adams attacks	+	+	+
3	17	Fatigue dizziness at rest	+	−	−
4	23	Periodic lightheadedness near syncope at rest	+	+	−

Table II *Training programmes*

Pat. no	Competition discipline	Type of physical activity	Duration of physical activity
1	League football	Long-distance running	1 h 3 times a week for 4 y
		Gymnastics	½ h 3 times a week for 4 y
		Football training	1–1½ h 3–4 times a week for several years
2	None	Crawling on a beach	2 h every day for months
		Swimming and gymnastics	1–1½ h every day for 2 y
		Walking exercises on crutches	Several hours every day for years
3	Motor-cross	Long-distance running or swimming	1 h every day for 3 y
		Gymnastics	2 h 5 d a week for 3 y
		Distributing newspapers	2 h every morning for 3 y
		Motor-cross training	1 h every day during week-ends several hours for 2 y
4	Karate	Long-distance running	1–2 h 4 times a week for 3 y
		Swimming	1 h 2–3 times a week for 3 y
		Karate training	2 h 4 times a week for 3 y

Table III *ECG findings and results of exercise tests*

Pat no	ECG: Sinus brady cardia	Wandering pace maker	LVH	Tall T waves	Other abnormalities	Exercise test: Max strain (kpm/min)	Max HR (beats/min)	Duration of bicycling (min)
1	+	+	+	+	Large abrupt changes of HR	1200	144	15
2	+	−	−	+	Periodic SA block 1st degree AV block incomplete RBBB	Not performed		
3	+	+	−	−	1st and 2nd degree AV block	1350	168	20
4	+	+	+	+	Large variations in HR	1350	188	21

RBBB = right bundle branch block

atrioventricular (AV) conduction are listed in Tables IV and V respectively

Bathmotropic as well as dromotropic function was depressed in all patients All had sinus bradycardia during rest Vagotonic maneuvres did not influence heart rate (HR) perceptibly

Large variations in HR during sinus rhythm were observed in patients 1 and 4 (Table IV) The former had abrupt changes of rate indicating shift between two separate pacemakers the latter had large phasic variations related to respiration In this particular patient extreme sinus bradycardia in

Table IV *Data on automatic activity of the heart*

Pat no	Rate of sinus rhythm (beats/min)	Rate of junctional escape rhythm (beats/min)	Maximal SAN recovery time (msec)	Maximal HR (beats/min)	
				After 1 mg of atropine	During exercise
1	37–56	38–40	3 760	73	120
2	59–61	–	1 440	75	Not performed
3	57–63	31–33	1 170	111	110
4	34–62	33–46	2 150	125	95
Normal value			<1 700		

Table V *Data on atrioventricular nodal function*

FRP functional refractory period ERP=effective refractory period

Pat no	AH interval (msec)	HV interval (msec)	Pacing rate producing 2nd degree AV block (beats/min)	FRP_{AVN} (msec)	ERP_{AVN} (msec)	After atropine	
						AH interval (msec)	HV interval (msec)
1	105	40	99	500	365	Not observed	
2	155	55	140	485	390	110	50
3	130	55	57	–	–	120 (1 1 conduction)	50
4	155	50	133	500	320	105	50
Normal values	65–130	35–55		330–500	230–390		

cluding a sinus pause of 4 780 msec appeared just after initiation of catheterization procedure In both patients pathological values of sinoatrio nodal (SAN) recovery time were obtained during incremental atrial pacing The effect of prolonged atrial pacing on SAN recovery time was estimated in patient 1 (Fig 1) Long pacing sequences made the SAN recovery time increase

Sinoatrial (SA) block was observed in patient 2 just after admission to hospital but the His bundle study did not reveal any signs of conduction disturbance within the SAN

Administration of 1 mg of atropine i v was followed by a 30–100% increase in HR and the abnormal variations in patients 1 and 4 were abolished Exercise during the His bundle study caused an acceleration of HR by 53–115% in the three patients who were able to exercise

A wandering pacemaker was observed periodically in the ECG of patients 1 3 and 4 at rest Intracavitary electrograms revealed that the phenomenon reflected interference between sinus rhythm and a junctional escape rhythm

The ectopic pacemakers were accelerated by atropine prior to the SAN of patients 1 and 4 causing junctional escape rhythms of 60 and 80 beats/min shortly after the injection

Atrioventricular nodal (AVN) conduction was variously affected (Table IV) The atrio-His (AH) intervals of patients 2 3 and 4 were prolonged during sinus rhythm and all AVN refractory

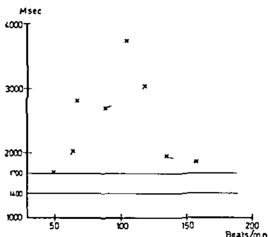


Fig 1 SAN recovery time in patient 1 Upper curve obtained after long pacing sequences lower curve after pacing sequences of 20 sec duration

periods determined tended to reach the upper limit of the normal range Intermittent second degree AV block was observed during sinus rhythm in patient 3 and a rate dependent AV block appeared at abnormally low pacing rates in patients 1 and 4

Injection of 1 mg of atropine promptly normalized the AH intervals, and the second degree AV block of patient 3 disappeared for a period of 129 min

The intraventricular conduction time represented by the His ventricular (HV) interval was normal in all four patients and was unaffected by atropine

At the re examination all patients did well They were in regular sinus rhythm still had a tendency to low HR but no arrhythmias Exercise tests were tolerated well, and the physical condition of the patients was excellent as before

DISCUSSION

The relative bradycardia which was found in all our four patients is a constant finding in well trained persons (1 5–9) It is due to increased vagal tone the mechanism of which is incompletely under stood Most probably it is an example of true re ciprocal excitation as proposed by Levy (10) ac cording to whom sympathetic impulses from work ing skeletal muscles can elicit the release of acetylcholine from postganglionic vagal fibers by virtue of the action of norepinephrine at some pre ynaptic α receptors

The strong vagal tone caused by training im roves the pumping capacity of the heart but dur ing rest the vagal preponderance may be so marked that SA or AV block appears (11) as in our patients *2* and *3* respectively or a sick sinus node syndrome is mimicked (3 4) as in our patients 1 and 4

To differentiate between mere vagal depression of SAN and a true sick sinus node syndrome in which some structural changes are always present in addition to vagal preponderance determination of the SAN recovery time after rapid atrial pacing and observation of the response to i v administra tion of atropine are commonly used (2 4) In pa tients 1 and 4 abnormally long SAN recovery times were found especially after prolonged pacing At pacing rates higher than 120/min however a rate dependent entrance block impeded overdrive suppression of the node As the SAN function was normalized after atropine in both patients the dis turbance must be merely functional

In patient 1 not only automatic but also conduc tion activity were affected causing frequent shifts between two separate pacemakers both probably located within the SAN (4 13)

Impairment of the AVN was noticed in all four patients but only apparent in the surface ECGs of patient 3 who had second degree AV block at rest The AVN transmission time (AH intervals) was normalized after atropine in all patients In the junc tion area automatic activity appeared to be unaf fected or only slightly depressed by the increased vagal tone as escape junction rhythm readily in terfered with sinus rhythm creating wandering pacemakers in three patients In two of these the junctional pacemaker was accelerated by atropine at an earlier point of time than the SAN

From the present study it can be proposed that *the bradycardia of athletes may be an expression of* vagal preponderance which suppresses all automa tic and conductive tissue of the heart In some cases the suppression is strong enough to provoke long sinus pauses at rest accompanied by cerebral symptoms However the risk of such complica tions is confined to periods of very intensive train ing and abolished when training intensitivity is re duced

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Neutrophil Kinetics in Acute Bacterial Infection

A Clinical Study

Niels Ebbe Hansen Hans Karle and Niels Henrik Valerius

From Department of Infectious Diseases M and Division of Haematology Department of Medicine A Rigshospitalet Department of Medicine C Gentofte Hospital and Division of Haematology Department of Medicine Hvidovre Hospital University Hospitals of Copenhagen Denmark

ABSTRACT Neutrophil kinetics in peripheral blood were studied with $DF^{32}P$ labeled cells in eight patients during severe acute bacterial infection Contrary to previous studies in man, the blood transit time of labeled neutrophils was short and the neutrophil turnover rate increased, up to ten times the normal during the early phases of infection This early phase was followed by a period in which the specific neutrophil radioactivity in the blood remained constant for up to 50 hours, probably indicating that in early convalescence neutrophil egress from the bone marrow to the blood is almost stopped The demonstration of increased neutrophil turnover may seem to illustrate what might be considered an obvious fact, but is in contrast to previous findings and seems to obviate the prevailing theory of quantitatively unchanged but redistributed neutrophil kinetics during bacterial infection in man The mechanism which apparently abruptly stops neutrophil egress from the bone marrow to the blood during early convalescence is unknown

The kinetics of neutrophilic granulocytes in bacterial infection have been studied in man (1 6) and in the experimental animal (8) using radioactively labeled cells In man—contrary to what might have been expected—the intravascular neutrophil transit time was found to be prolonged and the neutrophil turnover rate either normal or only slightly increased (1 6) Since these findings seem inconsistent with the well known rapid and great accumulation of neutrophilic granulocytes at the site of infection a redistribution of neutrophils involving an opening of new channels of neutrophil egress from the blood to the site of infection combined with a closing of customary channels has been postulated (1 4) However such a complicated mechanism is difficult to understand and other pieces of evidence militate against the concept of an unchanged but redistributed neutrophil turnover rate thus the myeloid predominance in the bone marrow probably indicates increased neutrophil production and the theory is not supported by findings in the dog (8)

It was the aim of the present work to study neutrophil kinetics in the acute phase of bacterial infection in man We therefore focused on patients with acute bacterial meningitis since this is a severe infection with an often well defined time of onset which quickly brings the patient to hospital

PATIENTS AND METHODS

Clinical

Eight patients with infection were studied within the first 24 hours after admission relevant clinical data are given in Table I Five patients without infection and with normal haematological values were studied as controls (7) Prior to the present admission all patients had been in good health except patient 8 who had steatosis of the liver on the basis of alcoholism On admission the patients with meningitis were treated with penicillin sulphonamide and streptomycin Following etiological diagnosis of either pneumococcal or meningococcal meningitis streptomycin was discontinued The patient with pneumonia was treated with penicillin During the kinetic studies prednisone (10 mg four times daily) was given to two patients (nos 6 and 8) The severity of the disease was arbitrarily graded into light moderate and heavy on the understanding that all patients had a severe potentially fatal infection Three patients (nos 3 6 and 8) made a slow recovery over several days patients 3 and 8 developing delirium tremens Five patients made a quick recovery with a

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Abbreviations $DF^{32}P$=diisopropyl fluorophosphate CGP=circulating granulocyte pool TBGP=total blood granulocyte pool GTR=granulocyte turnover rate MGP=marginated granulocyte pool

Correspondence to Niels Ebbe Hansen M D Division of Haematology Department of Medicine Hvidovre Hospital DK 2650 Hvidovre Denmark

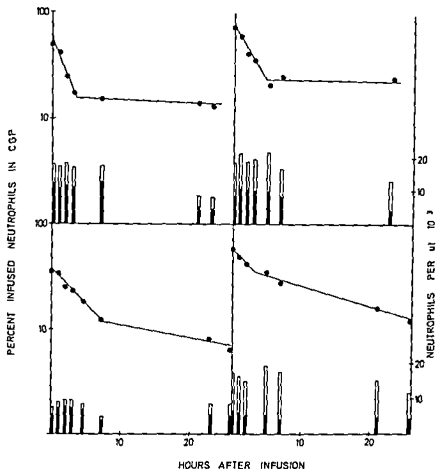


Fig 1 Neutrophil kinetic curves in patients 1–4 short term studies *Abscissa* hours after infusion of labeled neutrophils *Ordinate* neutrophil associated radioactivity in the blood as percent recovery after reinfusion Vertical bars show neutrophil counts in the blood (*right ordinate*) solid bars indicate number of immature neutrophils

definite improvement during the first 1–2 days following he start of antibiotic treatment All patients were dis ed in good health

oratory

eutrophil kinetics were studied with diisopropyl *fluorophosphate (DF³²P) labeled cells as previously de*scribed in detail (7) In short the procedure was as follows 500 ml blood was drawn into a plastic bag and incubated with 30 μCi DF³²P (Amersham England) for 45 min the total amount of DF³²P given varied between 0 17 and 1 1 mg After rapid reinfusion blood samples were drawn at the times indicated in Figs 1–3 As the main purpose of this work was to study the initial phase of bacterial infection frequent sampling was carried out during *the first 24 hours of the study However in addition to* this neutrophil radioactivity was followed for several days in four patients Leucocytes were isolated as previ

Table I *Patient data*

Pat no	Sex	Age (y)	Diagnosis	Duration of disease before study (d)	Antibiotic treatment before study (h)	Severity of disease
1	♂	20	Meningococcal meningitis	2½	20	Light
2	♂	20	Meningococcal meningitis	2	21	Light–moderate
3	♂	66	Pneumonia	2	23	Moderate
4	♂	41	Meningococcal meningitis	3	21	Moderate
5	♀	23	Meningococcal meningitis	3	29	Light
6	♂	39	Pneumococcal meningitis	1	18	Moderate–heavy
7	♂	19	Meningococcal meningitis	1	8	Light–moderate
8	♂	55	Meningococcal meningitis	3	20	Moderate–heavy

Table II *Kinetic data*

Pat no	T/2 (h)	Neutrophils (per µl)	CGP (fractional)	TBGP (×10⁷/kg)	GTR (×10⁷/hour)
1	2	16170	0.50	225	86
2	2¼	15780	0.75	164	42
3	4	20520	0.58	170	31
4	4	7100	0.35	115	19
5	2¾	7700	0.43	95	21
6	3	17750	1.00	123	29
7	7	19670	1.00	140	14
8	12	15750	0.89	101	6
Reference values					
Present study range	6.0–6.8		0.24–0.52	36–76	4–8
Bishop et al (2)					
Mean	6.3		0.44	61	7
95% range	4–10		0.2–1.0	27–138	3–17

ously described (7) and neutrophil associated radioactivity was counted in a liquid scintillation counter The cpm per 10^8 neutrophils were plotted against time in a semilogarithmic plot and from the initial slope (Figs 1–3) the T/2 (hours) was calculated using computer assisted regression analysis The blood volume of the patients was measured with T 1824 and the hematocrit values

In addition to the T/2 the following kinetic variables were calculated (In conformity with generally accepted kinetic nomenclature the designation granulocyte= neutrophilic granulocyte has been maintained)

CGP (per kg)=circulating granulocyte pool=no of neutrophils/µl blood × blood volume

Fractional CGP=

$$\frac{\text{total neutrophil radioactivity in CGP (10 min after infusion)}}{\text{total neutrophil radioactivity infused}}$$

TBGP (per kg)=total blood granulocyte pool=

$$\frac{\text{CGP}}{\text{fractional CGP}}$$

GTR (per hour)=granulocyte turnover rate=

$$\text{TBGP} \times \frac{\ln 2}{\text{T/2}}$$

Total leucocyte and differential counts were performed on all blood samples as shown in Figs 1–3 As immature cells were counted myelocytes metamyelocytes and stabs myelocytes constituted 1–2% in five patients 5% in two and 7% in one patient

RESULTS

The kinetic curves and the blood neutrophil counts from all patients are shown in Figs 1–3 It appears from these that during the initial phase—i e at least during the time of the first steep slope—the neutrophil counts in the blood and the degree of immaturity were in a steady state during the long term studies the neutrophil counts showed a slight decrease

It appears from the kinetic curves that all patients had two component disappearance curves made up of an initial fast disappearance followed by an almost horizontal line with virtually no change in neutrophil radioactivity Among these patients the initial T/2 was short (6/8) or normal (1/8) being prolonged in only one case (no 8) The latter was also the only patient with a medical history prior to this admission (chronic alcoholism and steatosis of the liver) and he was treated with prednisone during the time of study In the long term studies it is seen that the horizontal lines continued up to about 40–50 hours and were followed by a decline towards background radioactivity which was reached at 60–70 hours In contrast all five controls had monoexponential disappearance curves and T/2 values as demonstrated in other normal series

The kinetic variables shown in Table II were calculated from the initial rapid neutrophil disappearance curves A steady state during these few hours was assumed on the basis of the constant neutrophil counts and constant degree of immaturity It is of interest that the GTR in all patients but one (no 8) was higher than in the normal controls the maximum values being as high as ten times the reference mean

It was not possible to demonstrate any correlation between kinetic results and such variables as

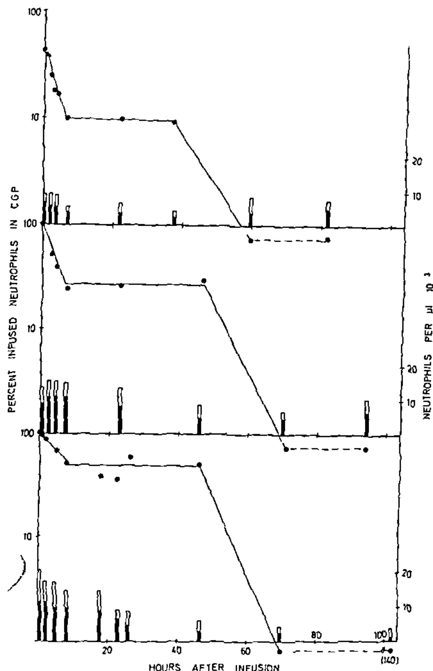


Fig 2 Neutrophil kinetic curves in patients 5 6 and 7 long term studies Symbols as in Fig 1

infecting microorganism time of onset of disease before study severity of disease or previous history except possibly in patient 8 in whom alcoholism liver disease and prednisone treatment cannot be excluded as being of significance in producing the slow T/2 and low GTR

DISCUSSION

In the present experimental set up a decrease in the specific neutrophil radioactivity in the blood results from input of unlabeled cells from the bone marrow which dilutes the blood neutrophil radioactivity whereas egress of neutrophils from the blood to the tissues presumably will affect labeled and unlabeled cells to an equal degree thus inducing no change in the specific neutrophil radioactivity

Since neutrophil counts and the degree of neutrophil immaturity were rather constant during the first steep slope indicating an approximately steady state it seems safe to conclude that the

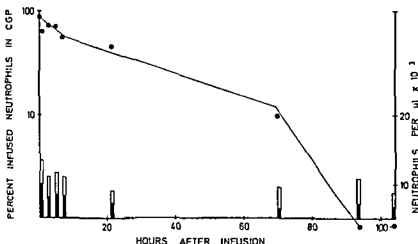


Fig 3 Neutrophil kinetic curve in patient 8 with steatosis of the liver due to alcoholism Symbols as in Fig 1

neutrophil turnover rate was usually greatly increased at this time of the study although the GTR values shown in Table II may be approximations This demonstration of a greatly increased GTR early in severe bacterial infection may explain how a great number of neutrophilic granulocytes is made available for the site of infection and there seems to be no need for a theory of redistribution of neutrophils in these patients Furthermore this study has demonstrated a kinetic pattern not previously recognized viz the combination of a short T/2 together with neutrophil leucocytosis and increased TBGP the main rule in neutrophil kinetics being that an increased TBGP is associated with a prolonged T/2 (1)

These findings differ from those of previous reports on neutrophil kinetics in bacterial infection in man (1 6) which have demonstrated a prolonged T/2 and a normal or only moderately increased GTR These differences are probably due to the fact that our patients were studied at an earlier phase of their infection than the patients in the previous studies who—according to the authors—had subacute or chronic infection The greatly increased GTR in early infections is actually also better in line with the myeloid predominance of the bone marrow seen in bacterial infection Possibly in severe bacterial infection the bone marrow comes dangerously close to complete depletion before increased myeloid proliferation can compensate for the increased consumption In fact a ten fold increased GTR equals the turnover of 10^{12} cells per 24 hours in a normal adult This is ten times more than can usually be accomplished with granulocyte transfusions from cell separators

It is a remarkable fact that a very similar kinetic pattern was found in all our patients but one (no 8) The different findings in the latter patient could possibly be attributed to his chronic alcoholism which might be responsible for a diminished bone marrow neutrophil depot it is also possible that the prednisone given to this patient attributed to the prolonged T/2 which is a well known effect of prednisone although the only other patient (no 6) who also received prednisone during the study did not show this kinetic pattern

A second interesting result of the present study is the finding of an almost horizontal line of neutrophil radioactivity in most patients after the initial steep slope Since a decrease in blood neutrophil radioactivity is dependent upon input of unlabeled neutrophils from the bone marrow the demonstrated constant neutrophil radioactivity in this phase of the infection probably means that the output of neutrophils from the bone marrow is almost stopped either prior to or simultaneously with clinical improvement The slow decrease in blood neutrophil counts indicates a small scale output of neutrophils from the blood to the tissues This feature of neutrophil kinetics has not been demonstrated in previous studies in man (1 6) but seems to parallel results from kinetic studies performed during convalescence from induced bacterial infection in the dog (8)

Although the present study was designed to study the initial phases it does appear from the curves

followed for several days that the period of unchanged neutrophil radioactivity continued for about 40–50 hours after infusion after which it fell to background values Thus it appears that this unique situation may continue for a rather long time The mechanism of the late fall after 40–50 hours is unknown but might well be due to neutrophil death from senescence It has previously been suggested that neutrophilic granulocytes in the blood die from senescence after 30 hours (5) The possibly longer life span in this study could be due to the fact that in the present situation blood neutrophils were younger than is normally seen since presumably they have not spent several days in the bone marrow depot before reaching the blood

As first shown in the fundamental studies by Vejlens (9) and later confirmed in kinetic studies with radioactively labeled cells (8) bacterial infection leads to an increased margination of neutrophils to the vessel wall As can be inferred from Table II this could not be demonstrated in the present study the marginated granulocyte pool (MGP) constituting 0–65 % of the TBGP which is entirely within the reference range Since an increased MGP has been found to be a very early occurrence in bacterial infection (8) it is quite possible that this may have been a feature in our patients before they were available for the study

It should be emphasized that whereas an increased MGP would be revealed by a low recovery of neutrophils immediately following reinfusion the tribution of cells between the two vascular pools no bearing on the slope of the disappearance es which are determined on the basis of relative neutrophil radioactivity in the CGP It is a fundamental prerequisite for kinetic analysis that the two vascular neutrophil pools are in a dynamic equilibrium with no preferential compartmentalization of certain cells That this is really the case was observed already by Vejlens (9) and has been amply comfirmed

In conclusion this study has demonstrated a kinetic pattern of short blood neutrophil passage time and increased GTR in early bacterial infection This appears to obviate the theory that bacterial infection alters the generalized random loss of neutrophils from the blood to the tissues to the effect that the infectious site is favored at the expense of losses via normal channels of neutrophil egress The intriguing question of how the apparently rather prompt cut off of neutrophil release from the bone marrow to the blood in early convalescence which was demonstrated in animal experiments (8) as well as in the present study is brought about remains to be answered It would seem that kinetic studies in acute bacterial infection with the pronounced and sudden changes may provide answers as to regulatory mechanisms governing neutrophil turnover

The finding of greatly increased neutrophil turnover rates early in infection may call for a reconsideration of the number of neutrophilic granulocytes which should be given to infected neutropenic patients treated with neutrophil transfusions and may also explain the very low increments in blood neutrophil counts observed after granulocyte transfusions

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Oesophageal Disease Revealed by Endoscopy in 1 000 Patients Referred Primarily for Gastroscopy

Jean Cronstedt Lasse Carling Peter Vestergaard and Jonas Berglund

From the Department of Medicine Bollnas Hospital Bollnäs Sweden

ABSTRACT Of 1 000 patients referred primarily for gastroscopy, almost 18 % had endoscopic signs of clinically important oesophageal disease Erosive oesophagitis a disease that is notoriously difficult to diagnose on X ray was demonstrated in almost 10 % and oesophageal varices in 3 6 % of the patients It is concluded that a thorough examination of the oesophagus should be included in every routine upper gastrointestinal endoscopy

Reflux oesophagitis is one of the most treacherous diseases encountered in gastroenterology because of its troublesome complications James Roth 1974

The oesophagus undoubtedly occupies a leading anatomical position in the gastrointestinal tract Interestwise however doctors as a rule have placed it at the rear end of this organ system Thanks to front viewing and oblique viewing fibreoptic endoscopes examination of the oesophagus has now been made possible as part of the routine upper gastrointestinal endoscopy which has gradually replaced the more limited endoscopic examination with side viewing instruments of the stomach alone The purpose of this paper is to present the oesophageal findings in 1 000 patients referred primarily for an endoscopic examination of the stomach

METHODS

Except for a few emergency examinations all the endoscopies were performed after an overnight fast in the special endoscopy room of the Department of Internal Medicine by a skilled endoscopist Before the examination an ECG was taken an indwelling i v cannula was placed and special care was taken to explain the procedure to the patient Preparation of the patient also included the administration of 0 3–0 5 mg of atropine s c 10–15 ml of an antifoaming agent by mouth and the spraying of the pharynx with 4 % lignocaine The endoscopy was carried out under i v diazepam cover the average dose being 5–10 mg

All the examinations were performed in the left lateral position The instruments used were either an Olympus GIF K (oblique viewing) or an Olympus GIF D3 (front viewing) endoscope At no stage of the examination was more than gentle persuasive pressure applied to the instrument The oesophagus was examined on the way down and as soon as the stomach had been entered the gastric juice was removed by suction through the instrument in order to minimize the risk for aspiration Concluding the examination of the stomach and the duodenum retroversion of the instrument in the stomach was performed in order to facilitate visualization of the cardiac region and the fundus Finally re-examination of the oesophagus was carried out during the withdrawal of the instrument During the entire procedure blood pressure and pulse rate were recorded at regular intervals by an endoscopy assistant

After the examination the patients were allowed to recover under nursing observation in a recovery room or in the ward Thereafter the findings were explained to the patient by the endoscopist and a written report was sent to the referring doctor In order to avoid complications due to prolonged effects of the i v diazepam no patient was allowed to drive back home or to return to potentially dangerous work on the same day

PATIENTS

From March 5 1975 to Oct 10 1977 altogether 1 158 upper gastrointestinal endoscopies were performed in 1 000 patients They were referred for endoscopy by general practitioners in the province as well as by doctors in the Departments of Medicine and Surgery Bollnas Hospital and other nearby hospitals Most patients were out patients

RESULTS

Due either to inadequate technique of insertion or to organic obstruction attempts to pass the instrument into the oesophagus failed in 8 patients Normal endoscopic findings were demonstrated in 816 and endoscopic signs of oesophageal disease in 176 patients

Of the 95 patients with erosive oesophagitis 75 % were older than 50 and 66 % were men In half of the

Table I *Pathological endoscopic oesophageal findings in 1000 patients prevalence of hiatus hernia in 95 patients with erosive oesophagitis and associated endoscopic findings in 95 patients with erosive oesophagitis excluding hiatus hernia*

	n	%
Oesophageal findings		
Erosive oesophagitis	95	9 5
Oesophageal varices	36	3 6
Deep ulcers	10	1 0
Strictures rings webs	10	1 0
Benign polyps	9	0 9
Diverticula	6	0 6
Mallory Weiss tears	5	0 5
Malignant tumours	3	0 3
Total	176	17 6
Hiatus hernia		
Present	68	72
Not present	27	28
Total	95	
Associated findings		
No associated pathological findings	23	24
Erosive gastritis and/or erosive duodenitis	20	21
Duodenal ulcer disease	14	15
Gastric ulcer	10	11
Atrophic gastritis	7	7
Gastric retention	6	6
Oesophageal varices	5	5
Acute duodenitis without erosions	5	5
Coeliac disease	2	2
Gastric malignancy	2	2

36 patients with oesophageal varices these were of moderate to severe degree In 8 of the 10 patients with deep oesophageal ulcers these were located in the distal portion of the oesophagus Of the 10 patients with strictures rings or webs 4 had benign strictures in the distal and 3 in the middle portion 2 had a Plummer Vinson membrane and one a lower oesophageal ring Therapeutic dilatation was performed in 5 of these patients using the endoscope itself in 3 and Eder Puestow bougies in 2 Of the 3 malignant tumours 2 were adenocarcinomas in the distal and one squamous epithelial carcinoma in the uppermost portion of the oesophagus

Endoscopic signs of hiatus hernia were found in 145 patients 77 of these lacking signs of oesophageal disease The oesophageal lesions found the association between hiatus hernia and erosive oesophagitis as well as the associated endoscopic findings excluding hiatus hernia in the 95 patients with erosive oesophagitis are shown in Table I

Only one serious complication occurred A 65 year old man reacted with apnoea and cardiac standstill upon spraying the pharynx with lignocaine Immediate resuscitation was successful and he recovered completely A few weeks later endoscopy without prior local anaesthesia of the pharynx was performed without complications

DISCUSSION

Endoscopic examination of the oesophagus yielded signs of oesophageal disease in almost 18% of the patients erosive oesophagitis being the commonest disease encountered

Heartburn and retrosternal pain can occur in patients with endoscopically normal oesophageal mucosa (1) and has been shown to be an important manifestation of oesophageal dysfunction often leading to an erroneous diagnosis of angina pectoris (10) Mild oesophagitis is difficult to diagnose endoscopically since erythema depends on the degree of capillary dilatation in the papillae and the thickness of the epithelial layer (1) and since the eye of the examiner cannot ascertain the degree of infiltration by inflammatory cells Multiple complete erosions however are reliable signs of oesophagitis (3) In our series erosive oesophagitis was diagnosed only in the presence of multiple complete erosions or superficial ulcerations with or without patchy adherent mucous exudate Erythema and a friable mucosa alone were not considered to be reliable signs of oesophagitis No attempt was made to identify candida organisms by microscopic examination of direct smears from patches of exudate Thus we cannot exclude the possibility that some of our patients had candida oesophagitis a disease that has recently been suggested to be less uncommon than has hitherto been realized (4) Upon specific questioning after the diagnosis of erosive oesophagitis had been made only half of our patients gave a history of heartburn retrosternal pain or dysphagia

The true prevalence of oesophagitis in any population is largely unknown since attempts to diagnose this disease have been made in symptomatic or other highly selected groups of patients only Lodge (5) demonstrated oesophagitis in 8 of 100 patients who had died suddenly of various causes but in 38% of 300 cases who had died after varying periods of hospitalization often after prolonged bed rest indicating the association of oesophagitis with



Fig 1 Radiological appearance of an acute fulminating oesophagitis with stricture in a 65 year-old man

other diseases in general and with prolonged bed rest in particular In our 1000 patients the prevalence of erosive oesophagitis was almost 10% a figure that thus cannot be considered surprisingly high

Hiatus hernia was present in 72% of our cases with erosive oesophagitis Palmer (6) demonstrated such hernia in 45 5% of 413 patients with oesophagitis Turning it the other way around we found erosive oesophagitis in 47% of 145 patients with hiatus hernia whereas Palmer (7) demonstrated it in 25 7% *of 1000 symptomatic patients with such* hernia Pope II (8) however has emphasized that hiatus hernia is not an illness but an anatomic condition that may be misinterpreted as the cause of the patient s symptoms and thus may lead to unnecessary or wrong therapy To some degree his opinion seems to be refuted by the results of current research carried out by Tibbling (11) insofar as the occurrence of acute chest pain can be correlated to the protrusion of a hiatus hernia Nevertheless since erosive oesophagitis can now be readily diagnosed with routine upper gastrointestinal endoscopy we do think that this disease entity should be given much more attention in the diagnostic work up of patients with heartburn and retrosternal pain

It is important to diagnose oesophagitis since it can cause ulcer stricture bleeding and other troublesome complications (9) We found oesophageal ulcers in 10 strictures in 7 and haemorrhagic oesophagitis in 7 patients Figs 1 and 2 show the radiological and endoscopic picture of an acute fulminating oesophagitis that developed within 14 days of a prostatectomy For several years the patient a 65 year old man had been experiencing nocturnal heartburn at times but he had never consulted a doctor for his symptoms and he had never experienced dysphagia During the postoperative period he had been lying flat in bed and had received ampicillin capsules for 5 days for a wound infection Medical treatment did heal the ulcerations but did not cure either the dysphagia or the stricture and ultimately he had to be operated on His case illustrates how rapidly severe oesophagitis with stricture may develop in a patient with only moderate prior symptoms of oesophagitis

To our mind therefore it is imperative that all patients with oesophagitis whether symptomatic or asymptomatic whether diagnosed or just suspected do receive proper medical treatment When hospitalized all such patients should be placed in a bed that is properly tilted in order to prevent reflux Furthermore since capsules and tablets may become impacted in the normal oesophagus where they can cause severe ulcerations (2) all patients with diagnosed erosive oesophagitis should be told always to swallow their medicines with sufficient amounts of water

The association of erosive oesophagitis with other upper gastrointestinal diseases (excluding hiatus hernia) in 76% of our cases suggests complex *interrelations between oesophagitis on the one hand* and gastric and duodenal disease on the other hand It seems likely that the oesophagitic symptoms in many patients were overshadowed by symptoms caused by gastric or duodenal disease and that antacid treatment of such disease also had a beneficial effect on the oesophagitis

The other diseases of the oesophagus encountered in this series need fewer comments It should be pointed out however that of the 36 patients with oesophageal varices only 7 (19%) had known liver disease and that only an additional 9 patients gave a history of regular intake of alcoholic

beverages In 29 patients (81%) oesophageal varices were the first sign of portal hypertension Examples of oesophageal lesions found are shown in Figs 3–7

CONCLUSION

The high prevalence of oesophageal disease in patients referred primarily for gastroscopy emphasizes the importance of including a thorough examination of the oesophagus in every patient subjected to upper gastrointestinal endoscopy

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Fig 2



Fig 3



Fig 4



Fig 5

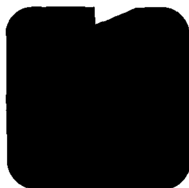


Fig 6



Fig 7

Fig 2 Acute fulminating oesophagitis with stricture Same patient as in Fig 1 *Fig 3* Severe ulcerative oesophagitis in a 75 year old woman with a history of occasional retrosternal pain *Fig 4* Moderate erosive oesophagitis with multiple complete erosions 55 year-old with heartburn for several years *Fig 5* Hiatus hernia in a 74 year-old man as seen with retroversion of the instrument in the stomach *Fig 6* Oesophageal varices in a 49 year old man with known cirrhosis of the liver *Fig 7* Polypoid carcinoma of the cardiac region in a 86-year-old man

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The Shock Liver

Clinical and Biochemical Findings in Patients with Centrilobular Liver Necrosis Following Cardiogenic Shock

Henrik S Birgens Jens Henriksen Peter Matzen and Hemming Poulsen

From the Departments of Internal Medicine (Division of Hepatology) Clinical Physiology and Pathological Anatomy Hvidovre Hospital University of Copenhagen Copenhagen Denmark

ABSTRACT Five patients with severe heart disease developed cardiogenic shock of more than 24 hours duration As a sequela to the shock severe liver affection was demonstrated Serum aspartate aminotransferases and serum lactate dehydrogenases showed very high activities The prothrombin proconvertin index was reduced to less than 25% of the normal Four of the patients were jaundiced The condition gave rise to some differential diagnostic problems Liver biopsies were available from four of the patients and histological examination of an autopsy specimen of the liver was performed in each case The liver histology showed centrilobular necrosis and haemorrhage in all patients It seems that centrilobular fibrosis develops later in the condition The pathogenesis of this liver affection is probably hypoxic injury to the centrilobular areas of the liver lobule due to reduced liver blood flow

Key words Shock cardiogenic liver circulation liver diseases

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Slight liver affection is common in congestive heart disease with right ventricular failure It is thought to be caused by venous congestion and prominent features of the liver histology are dilatation of the central veins and sinusoidal engorgement (15)

Severe changes of the liver with centrilobular necrosis do occur in shock or acute left ventricular insufficiency with forward failure and are mainly known from older pathological anatomical literature (4 7 17) The clinical picture of jaundice and excessively raised serum aspartate aminotransferase (S-ASAT) and serum lactate dehydrogenase (S LDH) values associated with these morphological changes of the liver was described first by Killip and Payne in 1960 (11) and in recent years by Bloth et al ([illegible]) and Cohen and Kaplan (5)

We present here five patients who all had cardiogenic shock and centrilobular liver necrosis which gave rise to severe differential diagnostic problems between primary liver disease and secondary liver affection caused by shock We want to call attention to the clinical and biochemical features in these patients and to relate them to the pathological anatomical findings

PATIENTS AND METHODS

The five patients were all characterized by cardiogenic shock acute clinical and biochemical liver affection and centrilobular liver necrosis They were admitted to medical departments in different hospitals in Copenhagen in 1964–76 The case records have been reviewed with special emphasis on previous liver or heart disease previous blood pressure (BP) actual heart disease actual BP during the shock duration of the shock and the time that elapsed from the possible restoration of the BP to liver biopsy and autopsy

The biochemical methods used for testing the values of S-ASAT S-LDH serum bilirubin prothrombin proconvertin (PP) index and serum creatinine were the routine methods of the different hospital laboratories at the time the particular patient was admitted The units and reference values were therefore different but the figures have been converted to SI units Reference values are given in Table II

Liver biopsies performed by the Menghini technique were available in four of the patients Two of the biopsies were obtained immediately post mortem Liver tissues were available from the autopsies of all patients and were evaluated histologically by one of us (H P) Centrilobular necrosis centrilobular haemorrhage biliary stasis centrilobular fibrosis and sinusoidal dilatation have been noted and are graded 0 + ++ +++ signifying not present or present in a slight moderate or severe degree

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Abbreviations S ASAT=serum aspartate aminotransferase S LDH serum lactate dehydrogenase BP blood pressure PP prothrombin proconvertin AMI acute myocardial infarction

Table I *Clinical data on five patients with centrilobular liver necrosis following cardiogenic shock*

Case no	Age (y)	Sex	Former liver disease	Former heart disease	Actual heart disease	Former BP (mmHg)	Actual BP (mmHg)	Duration of low BP (h)
1	63	♂	Normal liver biochemistry 3 mo previously	Angina pectoris slight left and right sided heart failure	AMI	130/80	70/–	27
2	63	♀	Unknown	Left and right sided heart failure	AMI	180/80	90/–	30
3	73	♂	Unknown	Slight left sided heart failure	Cardiogenic shock no actual myocardial infarction	180/85	100/–	24–48?
4	67	♂	Slight steatosis 1 y previously	Angina pectoris left sided heart failure	AMI	115/85	70/55	45
5	58	♂	Normal liver biopsy 6 mo previously	Angina pectoris left and right sided heart failure	AMI	150/90	100/–	48

RESULTS

The clinical data on the five patients are summarized in Table I the biochemical findings in Table II and the results of the histological examination in Table III

SELECTED CASE REPORT

Case 2

A 63 year-old woman treated for 30 years for arterial hypertension Her BP was about 180/80 mmHg during atment For eight years treated with digoxin for atrial rillation

When 61 years old she was admitted for thoracic pain cardiac insufficiency and a fast perpetual arrhythmia BP was 120/70 mmHg ECG showed an atrial fibrillation and a left bundle branch block S ASAT was 6 50 µkat/l S LDH 12 2 µkat/l The diagnosis of acute myocardial infarction (AMI) was made and the patient was treated with digoxin diuretics lidocaine and procainamide Five days after admission she developed clinical shock with a systolic BP of 90 mmHg S ASAT rose to 117 33 µkat/l S LDH was 185 0 µkat/l of which 80% was due to the liver-correlated isoenzymes PP was spontaneously 0 10 On the next day when the BP was restored the patient was jaundiced and her renal function was slightly decreased She was transferred to a department of hepatology because acute fulminant hepatitis was suspected Hepatitis B antigen and antinuclear factor were not found After stabilization of the circulation the hepatic and renal functions improved A liver biopsy seven days after the shock showed small centrilobular necrosis with haemorrhage moderate bile stasis and no fibrosis

During the following two years the patient suffered from chronic congestive heart failure from which she finally died Liver biopsy had been repeated one and two years after the shock The biopsies showed a consistent picture with slight centrilobular fibrosis and moderate sinusoidal dilatation but no centrilobular necrosis (Fig 1)

Autopsy showed a hypertrophic heart with fibrotic myocardial scar The lungs and the liver were marked by chronic venous congestion The liver histology was dominated by sinusoidal dilatation

Table II *Biochemical findings*

Case no	S ASAT (µkat/l)	S-LDH (µkat/l)	S bilirubin (µmol/l)	PP index	S-creatinine (µmol/l)
1	490 67	–	53	0 16	582
2	117 33	185 0	146	0 10	189
3	154 00	297 3	293	0 14	483
4	50 67	–	–	0 23	349
5	15 13	33 2	352	0 18	268
Reference values	0 17–0 67	2 5–7 5	5–17	0 70–1 30	50–120



Fig. 1. Part of lobule from liver biopsy from case 2 showing sinusoidal dilatation and atrophy of the cell plates in the centrilobular area (right). Portal tract is seen on the left side. The biopsy was performed two years after the acute episode of cardiogenic shock. (H & E ×75)

DISCUSSION

No figures are available for the true frequency of centrilobular necrosis of the liver following cardiogenic shock (shock liver) in the period 1964–76 in the medical departments of the Copenhagen hospitals. From 1st Sept. 1976 to 31st Dec. 1977 330 patients with AMI were admitted to the Department of Cardiology at Hvidovre Hospital. Two of these (cases 4 and 5) developed shock liver. During the same 16 months 365 patients were admitted to the same hospital with jaundice, which in two of these (cases 3 and 5) was caused by shock liver. Centrilobular necrosis of the liver following cardiogenic shock therefore seems to be a rare complication to acute heart disease and a rare cause of jaundice, the frequency of both being about ½%.

Centrilobular necrosis of the liver is not specific for cardiogenic shock, but probably it is the reaction of the liver on hypoxia and decreased liver blood flow. Centrilobular liver necrosis is also observed in other conditions complicated by shock, i.e. septicaemia, acute pancreatitis, haemorrhage, peritonitis, and as a sequela to major surgery (6, 7). Characteristically the lesions also appear in connection

Table III. *Histological findings in liver tissue*

0=Not present. +=slight. ++=moderate. +++=severe

Case no.	Biopsy	Time elapsed since shock	Centrilobular necrosis	Centrilobular haemorrhage	Bile stasis	Centrilobular fibrosis	Sinusoid dilatation
1	Autopsy	3 d	+++	+	+	0	0
2	Needle	7 d	+	+	++	0	0
	Needle	1 y	0	0	0	+	++
	Needle	2 y	0	0	0	+	++
	Autopsy	2 y	0	0	0	+	++
3	Needle	15 d	+	+	+	0	0
	Autopsy	17 d	+	+	+	0	0
4	Post mortem needle	0 d	++	++	0	0	0
	Autopsy	0 d	++	+	0	0	0
5	Post mortem needle	16 d	++	++	++	+	0
	Autopsy	16 d	++	+	++	+	0



Fig 2 Part of lobule from liver biopsy from case 4 A large centrilobular necrosis is visible on the right side of the picture with partly pre served outline of the liver cells but with lysis of the liver cell nuclei In addition there is slight infiltration with neutrophils and histio-cytes and slight steatosis A portal tract is present on the left side The biopsy was *performed two days after the* onset of the shock (im mediately post mortem) (H & E ×75)

with steatosis in poisoning with carbon tetrachloride (9) In the isolated perfused pig liver (13 16) normal oxygen uptake is maintained when the hepatic venous oxygen tension is above 30 mmHg Below this value corresponding to an average reduction in hepatic blood flow of 70% the oxygen uptake the galactose elimination capacity and the adenosine triphosphate concentration of the liver are decreased and the lactate/pyruvate ratio of the hepatic venous blood is considerably increased These findings indicate that the liver is relatively sistant to decreased oxygen supply Reduction in xygen supply beyond a critical point is however incompatible with normal oxidative metabolism Rabøl and Pedersen (14) showed that a reduction to about 15% of the normal oxygen supply for 90 min *resulted in reversible ultrastructural changes in the* centrilobular hepatocytes of the isolated perfused pig liver Vacuolization of the cytoplasm and changes of the mitochondria were demonstrated Ellenberg and Ossermann (7) found that cardiogenic shock of less than 24 hours duration is unlikely to be followed by centrilobular liver necrosis whereas it regularly follows shock lasting more than 24 hours Although the liver blood flow is considerably decreased in forward failure (7 11) pathophysiological studies have shown that the human liver after lowering of the splanchnic blood flow for one hour by vasopressin is able to increase the oxygen extraction and therefore maintain a constant oxygen consumption within rather wide limits (10) This might explain why a short lasting decrease in cardiac output does not regularly result in centrilobular liver necrosis

All patients in this study had hypotension for more than 24 hours The relation of hypotension to the development of centrilobular necrosis is stressed by several authors (2 4 7 11) However Bang et al (1) had in their series seven patients with AMI excessively raised S ASAT and centrilobular necrosis in liver biopsies in which no correlation to hypotensive episodes could be demonstrated They assumed that the centrilobular necroses were caused by severe right sided heart failure Likewise Gadeholt and Haugen (8) have described a patient with severe jaundice due to centrilobular hepatic necrosis caused by rheumatic heart disease with *backward failure but without shock On the other* hand it is a general experience that the common liver involvement in chronic congestive heart failure is less dramatic with slight jaundice moderately elevated liver enzymes and dilatation of the central veins with sinusoidal engorgement in the liver biopsy This was found in our case 2 after the patient had recovered from the centrilobular necrosis but suffered from severe right ventricular failure (Fig 1) The histological picture with centrilobular necrosis and haemorrhage demonstrated immediately after the shock is quite different (Fig 2) Bile stasis was present in four cases and as a later development centrilobular fibrosis was demonstrated in two patients 16 days and one

year respectively after the shock The significance of these findings is unknown The different morphological findings have been evaluated semi quantitatively It is noteworthy that the degree of the changes varies so much but it was impossible to demonstrate any correlation to clinical and biochemical parameters

The extremely high activities of S ASAT and S LDH up to a hundredfold the upper normal limits are probably due to enzymes of liver origin because they are much higher than those usually seen in patients with even large myocardial infarctions (3) This was confirmed in two of our patients in whom isoenzyme determinations of S LDH demonstrated that the high S LDH activities were mainly due to isoenzymes of hepatic origin All patients had a substantial spontaneous drop in PP to less than 25% of the normal value indicating a poor liver function This has not been described before but Killip and Payne (11) noted an increased sensibility to coumarin in four patients with primary heart disease and centrilobular liver necrosis

The prognosis of this acute liver affection seems to depend primarily on the condition that caused the shock The changes in the liver function and morphology are supposed to be at least partly reversible as demonstrated in cases 2 and 3 The reversibility of the changes in liver morphology is probably due to the fact that the reticular framework of the necrotic parts of the liver lobule persists after the necrosis so that it serves as a template for regeneration In experiments with rabbits in which shock was induced the animals developed centrilobular liver necrosis and signs of regeneration were found 48 hours later (12) There is no specific treatment of the condition apart from treatment of the underlying heart disease The renal insufficiency seen in all our patients may be an indication for dialysis

CONCLUSION

Based on five case studies on patients with centrilobular necrosis following cardiogenic shock and a review of the literature we conclude that shock liver is a rare complication to acute heart disease which should not be confused with the common liver affection in chronic congestive heart failure or acute fulminant hepatitis

The clinical picture can be characterized as follows 1) There is a primary heart disease complicated by cardiogenic shock of usually more than 24 hours duration 2) Jaundice develops within the following 24–48 hours 3) The liver enzyme activities in serum are excessively increased 4) The PP index shows a considerable fall 5) Renal insufficiency is regularly seen 6) The outlook for the patient depends on the prognosis of the primary heart disease

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Whipple's Disease

Clinical and Histopathological Changes during Treatment with Sulphamethoxazole Trimethoprim

Palle Tauris and Jørgen Moesner

From the Department of Medicine Silkeborg Centralsygehus Silkeborg and the Institute of Pathology Århus Amtssygehus Århus Denmark

ABSTRACT A case of Whipple s disease involving the small intestine, bone marrow and peripheral lymph nodes diagnosed one year after the initial arthritic symptoms by peroral jejunal biopsy and rapidly responding to treatment with sulphamethoxazole-trimethoprim is described The effectiveness of therapy was monitored by clinical evaluation of the patient's condition repeated intestinal biopsies, determination of body weight, faecal lipid excretion and haematological values Clear cut clinical remission was induced promptly, and after nine months treatment, the patient was in perfect health The drug was well tolerated and folate deficiency did not occur After four months of antibiotic therapy, bacterial capsular remnants could still be demonstrated in the bone marrow aspirate The characteristic small bowel mucosal PAS-positive macrophages visualized in the jejunal biopsy specimens, persisted in reduced number after nine months of treatment

This vagarious condition was described by Whipple in 1907 (27) Up to the septennial year of Whipple s original contribution eight cases had been recorded in this country (1 5 6 10 11 17) the first in 1945 (7) In six patients diagnosis was established by autopsy or during laparotomy whereas two cases were diagnosed by peroral jejunal biopsy (1 11)

In a review of the world literature published in 1974 Miksche et al included 238 cases of Whipple s disease (21) The first report on a histologically verified case diagnosed in a living patient appeared in 1947 (23)

Whipple s disease is a severe multisystemic disorder The main clinical features are abdominal pain diarrhoea steatorrhoea loss of weight arthritis and pigmentation of the skin Fever and chronic cough are both common Migratory intermittent non-deforming seronegative polyarthritis and polyserositis often precede the gastrointestinal symptoms by many years In a review of all cases reported in the English language through 1961 30% of the patients developed arthritis at least five years prior to diagnosis (18) and premonitory arthritis of 20 years duration has been described (9) In a few cases diagnosis was made by jejunal biopsy in patients without any definite intestinal disturbance In these patients pseudo-Addisonian cutaneous pigmentation hypocalcaemia or arthritis suggested the diagnosis (13) Miscellaneous presenting symptoms include peripheral lymphadenopathy hypotension cardiac involvement with pericarditis or brain involvement with neurological signs

Whipple s disease usually offers considerable diagnostic problems The clinical manifestations vary from case to case and a variety of tissues may be involved in the presenting symptoms or appear in an unpredictable sequence The average age of these patients at the time of diagnosis is 40 years 47% of the cases occur between the ages of 30 and 50 with a predominantly male predilection (12 18) The laboratory findings are those of steatorrhoea and other manifestations of the malabsorption syndrome such as anaemia low plasma albumin and low serum calcium

The disorder is considered to be infectious presumably associated with immunological defects especially impaired cell mediated immunity (14 24) Investigations of serum immunoglobulins have revealed inconsistent results most often slightly depressed or elevated immunoglobulin fractions A lysosomal defect has been postulated (19)

Prior to the use of corticosteroids and ACTH in the 1950s Whipple s disease always ended fatally (16) During steroid treatment obvious remissions were observed in several patients but the final outcome was still uniformly lethal (4 15) In the 1960s the systematic use of antibiotics in the treatment of



Fig 1 Peroral jejunal biopsy before treatment. Abnormal villi crowded with PAS-positive macrophages PAS ×98



Fig 2 Jejunal biopsy after six months treatment Normal morphology of villi but persistence of PAS-positivity in macrophages PAS ×98

ipple s disease started a new and successful peutic era (4 8) although attempts to isolate e causative infective agent from intestinal mucosa or mesenteric lymph nodes were negative

CASE REPORT

Clinical data

Male 29 years admitted in Oct 1975 with intermittent pains located to the larger joints of the lower extremities and symptoms of a non-venereal urethritis The diagnosis of Reiter s disease was considered but not confirmed The *symptoms disappeared spontaneously and the patient was dismissed without any treatment In the course of six* months however the pains became permanent and fever attacks were present X-ray examination of the joints was normal and the only abnormal laboratory value was a high uric acid level During treatment for ten days with probenecid the pains decreased but the patient developed abdominal pains continuous fever peripheral lymph adenopathy fatigue and poor appetite

On readmission one year after the onset of the arthritic symptoms the patient had lost 8 kg and during the first week of observation diarrhoea occurred The patient suffered from severe abdominal pains malaise and extreme fatigue Biopsy of an enlarged axillary lymph node showed a diffuse hyperplasia with scattered groups of large histiocytes interpreted as suggestive of viral infection Anaemia and hypoalbuminaemia were present serum iron and serum cholesterol levels were very low (Table I) No pathogenic strains of Salmonella Shigella Escherichia coli Yersinia enterocolitica or enterovirus were found Barium meal studies of the entire gastrointestinal tract were normal Adaptometry revealed hemeralopia, and malabsorption was demonstrated by abnormal findings in D-xylose absorption test and increased faecal lipid excretion *The history suggested the diagnosis of Whipple s disease which finally was confirmed by peroral jejunal* biopsy showing coarse villi of uneven size crowded with macrophages containing granular strongly PAS-positive material characteristic of Whipple s disease (Fig 1) Histological reexamination and PAS staining of the initially only routinely stained lymph node biopsied one month prior to diagnosis revealed PAS-positive granules in the histiocytes

Table 1 *Data on the patient before and after treatment with sulphamethoxazole trimethoprim*

	Normal range	Months before treatment				Months after treatment			
		2	1	½	0	1	3	6	9
Hb (g/100 ml)	>12 0	14 0	11 0	10 4	8 5	8 0	12 2	13 3	14 0
ESR (mm/h)	<15	18	45	13	15	76	7	9	8
Leucocytes ($10^3/\mu l$)	4 7	8 2	5 4	7 9	5 7	7 2	6 4	7 2	
Thrombocytes ($10^3/\mu l$)	200–400		252		159	416	395	400	396
Eosinophiles (no /µl)	75–400		206	393	250	1 116	281	208	160
Reticulocytes (%)	<10		2		2	24	26	9	6
Erythrocyte folates (nmol/l)	400–1 200				720		940		865
S-albumin (g/l)	38–50	42	31	25	26	29	43	48	48
S-iron (µg/100 ml)	65–175		76		9	27	43		68
S-cholesterol (mg/100 ml)	150–300			90	97		215	285	
Faecal lipid excretion (g/24 h)	6–7			15	13	4			
Body weight (kg)		75 6	63 0	62 8	61 0	62 8	69 0	73 2	74 0
Stools (no /24 h)		1	2–3	6–7	10–12	1–2	1	1	1

Treatment

Therapy was instituted with tablets containing 400 mg sulphamethoxazole and 80 mg trimethoprim two tablets twice a day After a few days s medication the patient s condition improved rapidly After one month of treatment an attempt to reduce the dosage to 50% was immediately followed by relapse of symptoms with fever and abdominal pains as the dominating features The primarily instituted medication was then resumed for the rest of the nine months of treatment

Follow up examinations

A jejunal biopsy after six months treatment showed normal size and shape of the villi No significant reduction in the number of PAS-positive macrophages was evident (Fig 2) Simultaneous investigation by the immunoperoxidase technique (26) showed an abundance of plasma cells mainly IgA-containing in the lamina propria (Fig 3) These were virtually absent in the pretreatment biopsy After nine months treatment a diminished number and reduced staining intensity of PAS-positive macrophages was seen in a biopsy specimen from the jejunal mucosa (Fig 4) Six months after diagnosis the patient was well and on out patient control three and six months later he was found to be in perfect health The patient s data before and during treatment are shown in Table I

To detect a possible folate deficiency during long term treatment erythrocyte folate levels were measured before and during the medication period Folate deficiency did not occur and the drug was well tolerated



Fig 3 Immunoperoxidase reaction on the same biopsy specimen as in Fig 2 showing IgA-containing plasma cells (black) and a cluster of unstained macrophages (upper right corner) Counterstained with haematoxylin ×246

DISCUSSION

Whipple s disease seems to be closely related to the rheumatic or collagen diseases In a disease which used to be universally fatal complete and sustained

recovery following prolonged antibiotic treatment argues strongly in favour of an infectious nature The underlying lesion remains uncertain and the immunopathology of Whipple s disease which may hold the key to its pathogenesis still awaits elucidation

A large number of antibiotics in various combinations has been recommended in the therapy of Whipple s disease (10 11 20 21) Since the choice of therapeutic substances is based solely on empiric grounds and the sample sizes from different institutions are too small to allow any safe conclusion as to the effectiveness of the drugs involved it seems reasonable to choose a medication without side effects considering that long term treatment is necessitated Therapy with sulphamethoxazole trimethoprim has been used previously in the treatment of Whipple s disease without any side effects being observed (11)

In our case therapy provoked a flare up of transient fever a rising ESR and massive eosinophilia (Table I) This Herxheimer type of reaction vanished in less than two weeks

Some investigators suggest that bone marrow biopsy might be used diagnostically in suspected cases of Whipple s disease (2 25) The normal bone marrow—and lymph node macrophages however may contain PAS positive inclusions and distinction is unequivocal only on electron microscopy Yet there is no condition other than Whipple s

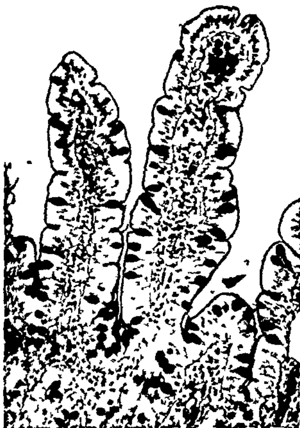


Fig 4 Jejunal biopsy after nine months treatment Diminished number and PAS positivity of macrophages PAS ×98

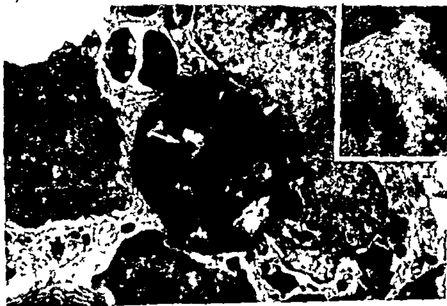


Fig 5 Electron microscopy of bone marrow aspirate after four months therapy Remnants of bacterial capsules (indicated by arrows) in macrophage lysosome Unstained ×8 400 (insert ×34 000)

disease in which the lamina propria of the small intestine is densely infiltrated with PAS positive macrophages (20) The easiest way of making the diagnosis therefore still seems to be peroral jejunal biopsy

The importance of diagnosis intra vitam is emphasized by the results of the antibiotic treatment and the paramount significance of small bowel biopsy in malabsorption suggestive of Whipple disease without recourse to laparotomy is thus stressed The present case also underlines the value of PAS staining in cases of non characteristic lymph node histiocytosis Although not diagnostic a positive result may offer an important clue to the diagnosis

The well known persistence of PAS positive macrophages through several months of treatment is due to slow degradation of the bacterial capsular material in these cells (19 22) as demonstrated here electron microscopically in a bone marrow aspirate after four months of therapy (Fig 5) The depletion of IgA producing plasma cells in the pre treatment intestinal mucosa is in accordance with the findings of other investigators (3) We have not been able to explain the initial urethritis and the high uric acid level

Further studies on future cases will be necessary to determine the exact aetiology of this enigmatic disorder

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Thallium Intoxication Treated with Long-term Hemodialysis, Forced Diuresis and Prussian Blue

Robert Smith Pedersen Anders Schou Olesen Leif Gade Freund
Per Solgaard and Elfinn Larsen

From the Department of Nephrology Ålborg Sygehus Syd Ålborg and the Department of Chemistry Risø National Laboratory Roskilde Denmark

ABSTRACT A 56-year-old woman, who ingested 2 g of thallium sulfate, was successfully treated with long term hemodialysis for 200 hours during ten days combined with forced diuresis and Prussian blue The effect of the artificial kidney dialysis was determined by repeated analysis of the thallium concentration in the dialysis bath and in blood samples During the first 120 hours of hemodialysis, 143 mg of thallium was eliminated via the artificial kidney and 110 mg via the urinary tract The present case of acute thallium intoxication is the first in which long term hemodialysis has been used in the acute phase together with forced diuresis and Prussian blue The data obtained are compared to those obtained from cases treated with hemodialysis in the past It is concluded that treatment with hemodialysis should be considered as an important supplement to treatment with forced diuresis and Prussian blue in cases of thallium intoxication

Prussian blue is an effective antidote used in the treatment of thallium intoxication (1 6 9 12) It acts by exchanging potassium ions for thallium thus rendering thallium less adsorbable Increasing the diuresis causes a substantial increase in renal elimination (5 7 8)

Treatment with hemodialysis appears to have been attempted previously only seven times (2 3 5 8 10 11) The combined use of forced diuresis hemodialysis and Prussian blue has been attempted in three cases (2 5)

CASE REPORT

A 56-year-old woman was taken to hospital after ingesting about 2 g of thallium sulfate Following gastric lavage the patient was transferred to the hemodialysis unit Treatment with forced diuresis laxatives and Prussian blue was immediately initiated Prussian blue $K Fe (Fe (CN)_6)$ 4 g was given 4 times per day and forced diuresis was obtained by giving furosemide i v Treatment with hemodialysis was instituted 12 hours after the intake and continued for 200 hours during the next ten days On the first day only one artificial kidney—Cordis[1,5,8]—was used thereafter two artificial kidneys were connected in series Every second hour during the hemodialysis blood samples were removed before and after the artificial kidney and samples of the dialysis bath taken The artificial kidneys were changed when the pressure difference between the inlet blood to and the outlet blood from the artificial kidneys exceeded 80 mmHg

The thallium concentrations were measured initially in blood and dialysis samples primarily to ensure that the artificial kidney was effective and secondarily to follow its capacity Other samples were stored in a freezer for later analysis The blood (10 ml) was collected in glass vials without a stabilizing reagent The total amount of urine for every 24 hours was collected and the thallium concentration measured After hemodialysis the forced diuresis was maintained for another two days and treatment with Prussian blue for another seven days Blood samples were still analyzed for thallium The concentration was below the detection limit 3×10^{-9} g/ml

The patient was discharged after 3 weeks in hospital A slight hair loss and transient paresthesia were noted

Chemical analysis

Dialysis bath and urine A 1–5 ml sample was transferred to a centrifuge glass and made up to 5 ml with H_2O One ml 4 w/v aqueous solution of ammonium pyrrolidine dithiocarbamate (APDC) and 1 ml methylisobutylketone (MIBK) were added After 60 sec of shaking the solution was centrifuged for 15 min at 2 000 rpm

Blood A one or two ml whole blood sample was acid digested in a beaker with 5 ml conc HNO_3+0 5 ml conc $HClO_4$ The nearly dry residue was dissolved and transferred to a centrifuge tube with H_2O 2 ml phthalate buffer pH 2 5 was added and the solution made up to 5 ml with distilled water One ml APDC solution and 1 ml MIBK were added and the solution was centrifuged

Thallium in the organic phase was measured with flameless atomic absorption on a Perkin Elmer spectrophotometer model 305 B equipped with a HGA 70 graphite furnace and D_2 background corrector Ten μl MIBK solu

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Abbreviations APDC=ammonium pyrrolidine dithiocarbamate MIBK=methylisobutylketone

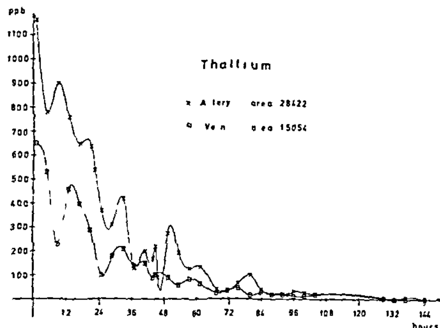


Fig 1 Tl concentration in the blood (10 ⁹ g/ml) before (artery) and after (vein) the artificial kidney versus time

tion was injected into the furnace and dried at 100°C for 30 sec ashed at 400°C for 15 sec and atomized at 2 000°C for 10 sec

Standard curves 50–500 ng Tl/ml MIBK were made by adding known amounts of Tl to pure dialysis water and urine samples with no Tl Batches of 10 samples were analyzed together with blanks and standards

RESULTS

Figs 1 and 2 show the concentrations of Tl plotted versus time in separate samples of blood and dialysis bath respectively The zero-time was midnight between Sept 4 and 5 1976 when the dialysis apparatus had been in function for almost 2 hours but there are no reliable data from that time The curves have been constructed by a Hewlett Packard computer with printer using a third degree spline function to fit the points (4) The thallium concentrations show an exponential decrease Thus after 2 days of hemodialysis the thallium concentrations in the blood had decreased by about a factor of 10 and after 5 days only negligible quantities

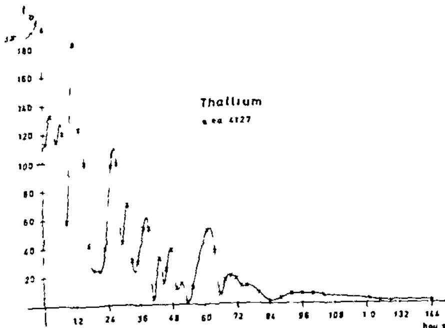


Fig 2 Tl concentration in the dialysis bath (10 ⁹ g/ml) versus time

Table I *Thallium in urine*

Period in Sept 1976	Concentration of Tl (μg/l)	Urine collected over 24 h (l)	Total amount of Tl (mg)
4–5	25 000	4.5	113
5–6	9 500	3.1	29
6–7	3 810	6.4	24
7–8	1 930	4.1	8
8–9	1 070	4.3	5
11–12	233	10.2	2.4
12–13	162	5.0	0.8
13–14	52	17.3	0.9

were found. Assuming a constant flow of blood —170 ml/min—and of dialysis bath—600 ml/min —through the artificial kidney, the areas under the curves represent the total amount of Tl in the blood and in the dialysis bath. Fig. 1 shows that 136 mg were removed from the blood during the first 120 hours of hemodialysis, agreeing well with the 149 mg Tl calculated from Fig. 2 to be found in the dialysis bath.

Calculating $C_A - C_V/C_A$ for the artificial kidney where C_A=the concentration of Tl in the artery and C_V=the concentration of Tl in the vein, a value of 0.49±0.15 was found for the range investigated (C_A=1 100–40 ppb). This corresponds to an average clearance of 83 ml/min.

The content of Tl in the collected urine samples is given in Table I. Generally, the different fractions of urine were collected over 24 hours. We estimate that of the 113 mg Tl removed from 4th to 5th Sept. 70 mg Tl was extracted before the start of hemodialysis. This implies that about 110 mg Tl was extracted via the urine in the first 120 hours of hemodialysis and about 5–10 mg Tl in the following 110 hours. After the withdrawal of furosemide only negligible quantities were found in the urine. The total amount of Tl removed via the urine was about 195 mg. Fecal elimination was not measured.

DISCUSSION

The patient in question had ingested 2 g of thallium sulfate corresponding to 1.6 g of thallium. The elimination during 10 days of treatment was about 335 mg via the artificial kidney and via the urinary tract. This corresponds to 21% of the thallium ingested.

In two cases reported by Jax et al. (5) it was possible to extract 17–29% of the ingested amount by treating with hemodialysis for only 8 hours a day during two respectively three days, combining this treatment with forced diuresis and Prussian blue. No information is given concerning the time that had elapsed between the intake and the initiation of treatment. Brittinger et al. (3) and Loew et al. (8) found the artificial kidney to be more effective than forced diuresis when treating thallium intoxication with long term hemodialysis and forced diuresis. Their patients were treated for 121 hours (3) and 77 hours (8) during 7 and 5 days, respectively. In the case reported by Brittinger et al. (3), more than half of the ingested amount could be removed via the artificial kidney and the urinary tract. This patient was not given Prussian blue, so one can assume that he had absorbed a greater part of the ingested amount than the two patients reported by Jax et al. (5) and the patient reported by us.

In a case reported by Barckow and Jenss (2) 65 hours had elapsed before treatment with hemodialysis for 54 hours was started. This patient was also treated with forced diuresis and Prussian blue. It was possible to remove 14% of the ingested amount via the artificial kidney.

The elimination can be enhanced 2–6 times by treating thallium intoxication with forced diuresis alone (7).

In the present case, treatment with hemodialysis was started in the acute phase and continued for ten days combined with forced diuresis and Prussian blue. Before the hemodialysis started, 70 mg of thallium had been removed via the urinary tract. Then during the first 120 hours of hemodialysis it was possible to eliminate 143 mg of thallium via the artificial kidney and 110 mg via the urinary tract. The thallium concentration in the blood was already decreased by a factor of 10 after 2 days of hemodialysis.

Based on the experience reported in the past and on this report, it seems justifiable to conclude that treatment with hemodialysis should be seriously considered in every severe case of thallium intoxication.

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MODERN MEDICAL HISTORY

Twenty Years of Cardiac Pacing in Sweden

In October 1958 the first subcutaneously implantable cardiac pacemaker was implanted by Senning at Karolinska sjukhuset in Stockholm (9) The introduction of transistors had made it possible for Elmqvist to construct a pulse generator small enough for implantation in the human body The energy of the first generator was obtained from nickel/cadmium cells The patient who was given the first generator had complete heart block with repeated Adams Stokes attacks He is still active and in good health and carries at present his 23rd generator

Prior to this first pacemaker implantation much work had been performed in other countries The first systematic work with heart stimulation using electrical impulses has been attributed to that of Albert Hyman who in 1932 stimulated arrested rabbit hearts (23) In 1950 Bigelow Callaghan and Hopps introduced an electrode to the heart through the jugular vein in dogs successfully stimulating their hearts in the neighbourhood of the sinus node With external electrodes and using an apparatus introduced by Zoll and Linenthal in 1952 heart stimulations through the chest wall became an accepted method of keeping human hearts with a tendency to stop beating (26) Stimulation of the heart with a stainless steel wire introduced into the myocardium after thoracotomy was successfully tried in a patient for the first time by Weirich in 1958 Two years later Chardack and Kantrowitz started to treat patients with pacemaker electrodes sutured to the heart after thoracotomy One year earlier Seymor Furman had introduced the transvenous route for insertions of pacemaker leads into patients via an antecubital vein External pulse generators were used initially

Epicardial electrodes were used when cardiac pacing was introduced at Karolinska sjukhuset in 1958 Because of threshold elevations and infections in some cases these electrodes were abandoned for routine use (8) The transvenous electrode was introduced for permanent pacing in 1962 (15) A series of 305 patients collected from different centra proved this method to be effective and made thoracotomy unnecessary (16) The transvenous route has however not been free from problems In a Swedish series of 260 patients paced in a mean period of two years with transvenous electrodes 31% developed complications necessitating surgery (5) The surgical technique has however improved and in the hands of trained surgeons the complication rate is at present not higher than a few per cent during the first year (10)

The main electrode problem besides early dislocations is threshold elevation Westerholm (25) studied different factors influencing the stimulation threshold and found the initial stimulation threshold to be of little significance for the chronic threshold Implantable pulse generators with facilities for threshold measurements have been constructed (20) making implantation of generators possible also in patients with high stimulation thresholds

Most paced patients have a temporary threshold elevation during the first months of pacing Fortunately this initial threshold elevation rarely exceeds the voltage of the pulse generator By decreasing the surface of the lead tip current density may be increased giving a lower threshold (24) However we still lack the ideal electrode which has a proven long life time and is simple to introduce into a stable position in the right ventricle

Soon after cardiac pacing was accepted as the method of choice for treatment of serious bradydysrhythmias it became clear to the treating physicians that many advanced heart blocks appear periodically (11) These experiences initiated research leading to construction of ventricular programmable pulse generators (ventricular synchronous and inhibited) Also the importance of atrial transport for optimal cardiac performance was demonstrated by Bevegård (1) and Bevegård et al (2) To synchronize atrial contractions with ventricular electrodes were sutured to the left auricular appendage necessitating thoracotomy (4)

An unfortunately high incidence of electrode dislocation made this procedure unsatisfactory An improvement occurred with introduction of a mediastinal detector electrode inserted by mediastinoscopy which has provided a more stable electrode for atrial triggered pacing (3) and these have been found to last for at least ten years (19) Especially patients with retrograde atrial activation and with decreased compliance of the myocardium as in aortic and pulmonary valve stenosis and cardiomyopathies have been shown to improve significantly with this stimulation technique (14 18) Although atrial triggered heart stimulation clearly is the most attractive mode of pacing there are obvious contraindications in patients with atrial arrhythmias

When initiating pacemaker treatment it is not possible to postulate if the conduction disturbance is permanent or not During the first period of cardiac pacing in Stockholm the permanently paced patients wore an external pulse generator during the first months (17) This policy made it possible to test different methods of stimulation such as fixed rate pacing ventricular synchronized pacing or atrial triggered pacing Today most patients are given ventricular inhibited pulse generators and the need for a two step procedure is therefore limited On the other hand it has become clear from the experience from the increased number of patients with intermittent heart blocks and the sick sinus syndrome who have been paced that some of them ve a real need for atrial transport As patients of category are not candidates for atrial triggered pacing the interest in atrial sensing and atrial stimulation or atrial sensing and ventricular pacing has increased Successful results with a transvenous electrode inserted into the right atrial appendix has recently been presented from a Swedish center (21)

Mercury cells have been the most often used energy sources in pacers Because of their limited life span attempts have been made to find other energy sources Nuclear cells i e plutonium batteries have been tried in several western countries In Sweden the National Board of Health and Welfare has not accepted this type of generators With the introduction of lithium cells which are long lasting chemical cells the need for nuclear cells has decreased considerably When pacing with implantable pulse generators was started 20 years ago the main problems were technical including electronic failure early battery depletion and electrode problems Even if these problems have not found their final solution they are small in comparison with those of the first period of cardiac pacing It is generally accepted that an expected life time of 5–10 years for lithium cells—dependent upon size—is realistic As most generators hitherto have been used for shorter periods we are still lacking experience concerning the durability of the electronic components over such periods Plutonium cells are an exception but the electronic components in them are essentially different from those in generators powered by chemical cells To be able to follow large series of implanted pacers and thus detect systematic failures the European Cardiac Society has initiated work with a computerized European register This central register is to be based on local registers in the different member countries

During the last few years a widening of the indications for cardiac pacing has evidently resulted in a steady increase in the number of paced patients An increased awareness of the sick sinus syndrome and introduction of new methods to reveal this syndrome mostly in the elderly with episodic symptoms but also pacing of patients with asymptomatic heart block are factors responsible for that increase Undoubtedly complete heart block infers an adverse prognosis even without syncope (12) The prognosis differs considerably between different individuals with complete heart blocks (6) We cannot however within this group define those —if any—for whom the prognosis is unaffected An important question remains how dangerous is the first Adams Stokes attack in a patient with complete heart block? Or expressed in another way should asymptomatic patients with grave conduction disorders be paced prophylactically because the first Adams Stokes might be the last?

With widened indications for cardiac pacing there has been an increased need for methods of selecting patients who really benefit from this treatment Telemetry Holter monitoring and intracardiac electrograms are methods that have still not found their definite place in the investigation of pacemaker candidates (13) An implantable pacer constructed for diagnosing episodes of extreme bradycardia or asystole has recently been presented (7) We hope that this generator will teach us more about the natural history of and the danger of having different kinds of conduction disturbances

Cardiac pacing has not only prolonged the life of

many patients (12) but has also provided a better quality of life (5) On the other hand some paced patients have symptoms—even disabling—because of lack of coordination between atrial and ventricular contractions They can be helped by changing the mode of stimulation At present we still lack simple methods which can help us to identify these patients Programmed pacers with components allowing for changes in stimulation rates as well as other characteristics after implantation have recently been introduced They are a step forward to a pacer with individualized stimulation possibilities

Olof Edhag Stockholm Sweden

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BOOK REVIEW

British Medical Bulletin Thrombosis vol 34 no 2 pp 101–212 £6 00 The British Council London 1978

The May 1978 number of the British Medical Bulletin Thrombosis complements the Bulletin published in Sept 1977 which was entitled Haemostasis The Haemostasis issue was of extremely high class The Scientific Editor of the Thrombosis issue is Dr D Thomas and he has provided an excellent introduction The Thrombosis issue comprises 14 papers by well known British authors in this field Also this issue is of high class but not as outstanding as the previous one This is probably because of our lack of basic knowledge of the thrombotic mechanism and the *treatment of thrombotic disorders which has also been* stressed by several of the authors

The issue starts with a paper by Dr Mitchell on Clinical Events Resulting from Thrombosis Formation The relationships between blood clotting factors platelets and thrombosis are dealt with in the first group of papers These papers are rather good but some information is lacking The role of the fibrinolytic activators in the vessel wall in the pathogenesis of thrombosis has not been considered sufficiently Furthermore recent observations indicate that factor XIIa is of importance for preventing thrombosis by mediating activation of endogenous fibrinolysis Patients with acquired inhibitors against factor XII thus have decreased fibrinolytic activity and a marked tendency to thrombosis Both Drs Davies and McNicol and Drs White and Heptinstall end their papers by stating that it is not yet possible to predict which individuals will develop thrombosis simply by studying blood components and in vitro platelet activity But inher deficiency of AT_{III} (antithrombin III) is one condition hich a tendency to thrombosis can be clearly defined ically Drs Barrowcliffe Johnson and Thomas ve written an excellent review of AT_{III} and heparin They pay special attention to the biological consequences of the heterogeneity of heparin and it is obvious that significant differences can be demonstrated in the effect on AT_{III} of heparins prepared from different tissues and of heparin fractions of varying molecular weights This opens up new therapeutical approaches in heparin therapy Dr Moncada and Dr Vane have written a very interesting chapter on "Unstable Metabolites of Arachidonic Acid and their Role in Haemostasis and Thrombosis" One of them PGI_2 is present in the vessel walls and its most important characteristic is that it is a highly potent inhibitor of platelet aggregation

Dr Woolf has a paper on a delicate problem namely the interaction between thrombosis and atherosclerosis

Problems of greater clinical interest are discussed in another group of papers Dr Browse has given a very useful and critical review of advantages and disadvantages of available methods for diagnosing deep vein thrombosis and pulmonary embolism The management of venous thromboembolism and also arterial thrombosis is dealt with in four papers The paper by Drs Morris and Mitchell on "Clinical Management of Venous Thrombo-Embolism" covers an important field in clinical medicine but is too superficial to serve as a general guide in this field Thus doses and different routes of administration of heparin in the treatment of manifest thrombosis (intermittent or continuous) are not discussed The effect of dextran is also commented only briefly The chapter by Drs Kakkar and Scully on thrombolytic treatment is well written The issue also includes contributions on blood clotting changes and thrombosis associated with the intake of oral contraceptives and the formation of thrombi on various artificial surfaces

This issue has been much more difficult to edit than the foregoing one on Haemostasis because the scope of the subject is so wide Though the issue does not cover the whole field it is a good review of the pathogenesis clinical manifestations and therapy of both venous and arterial thrombosis

Inga Marie Nilsson Malmö Sweden

Acta Med Scand 204 437–443 1978

"Non-Secretory" Plasma Cell Dyscrasia with Normal Serum Immunoglobulins

Leif E Wille Øystein Førre Per M Schjelderup Mathiesen
Torstein Hovig and Kåre Sorteberg

From the Department of Chemical Pathology Østfold County Hospital Fredrikstad the Institute of Immunology and Rheumatology and the Department of Electron Microscopy Rikshospitalet University Hospital Oslo and the Medical Department Halden Hospital Halden Norway

ABSTRACT A well documented case of non secretory myelomatosis with extensive skeletal lesions has been studied by immunohistologic techniques, electron microscopy, various electrophoretic proce dures and different studies of serum and urine In addition the various lymphocyte populations have been studied by immunofluorescent techniques rosette formation and stimulation with PHA and PWM mitogens The ultrastructural studies revealed a well preserved structure of most of the plasma cells often with a prominent nucleolus The Golgi ap paratus (GOL) was often large and in some cells there appeared to be a striking development of non granular (smooth) endoplasmic reticulum in associa tion with the GOL Various non-crystalline inclusions were seen outside the cisternae of the endoplasmic reticulum The cells contained large amounts of im munoglobulins of the IgG (kappa) class No parapro tein was present in serum Normal amounts of serum immunoglobulins were detected No abnormalities could be demonstrated in the B or T cell system It is concluded that the defect must be localized to the plasma cells themselves and not to the precursor cells of the B system

Key words Electron microscopy immunofluorescent studies lymphocyte function non secretory myelomatosis plasma cells

Acta Med Scand 204 437 1978

Multiple myeloma with plasma cells secreting intact immunoglobulins free light chains or free heavy chains usually represent no serious diagnostic problem By combination of clinical data marrow aspiration electrophoretic studies of serum and concentrated urine and immunodiffusion studies of serum immunoglobulins a fairly adequate diagnosis can usually be obtained

In the last decade however some reports have appeared in the literature on plasma cell dyscrasias without any detectable paraprotein in serum (5 21) In such cases the differential diagnosis against carcinomatous infiltration of the bone marrow from the prostate gland or pancreas can be difficult With the introduction of immunofluorescent techniques this diagnostic problem has been overcome in most cases However Gach et al (11) River et al (22) and Stavem et al (24) have reported on isolated cases with plasma cell dyscrasias in which specific marrow staining against the usual immunoglobulin chains could not be detected In the cases of Gach et al and Stavem et al ultrastructural studies con tributed to the differentiation between plasma cells and presumptive malignant cells

Kim et al (14) described five patients with multi ple myeloma in whom no paraprotein could be de tected in serum The clinical courses of all their five patients demonstrated progressive skeletal in volvement They also observed that these patients had a better survival than patients with multiple myeloma and serum paraproteins Hobbs (12) on the other hand suggested that this type of myelomatosis was the most dedifferentiated hav ing the poorest prognosis In all cases hitherto re ported severe reduction of normal serum im munoglobulins was the rule

We report on an atypical case of non secretory plasma cell dyscrasia in which a normal synthesis of immunoglobulins still is preserved In addition to conventional immunological studies we have car ried out special studies of the plasma cells including immunofluorescent staining electron microsco py identification of B and T lymphocytes phy

Abbreviations PHA=phytohemagglutinin PWM=poke weed mitogen GOL=Golgi apparatus GER=granular endoplasmic reticulum SER=smooth endoplasmic retic ulum

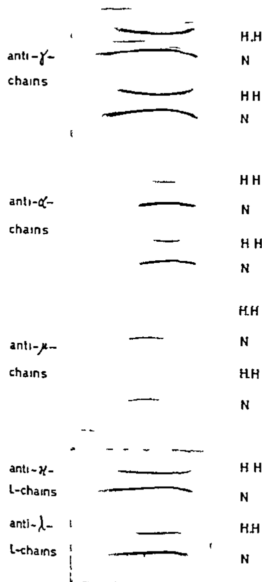


Fig 1 Immunoelectrophoresis (all regions in duplicate) of the patient s serum (*H H*) demonstrating no monoclonal protein and normal levels of IgG and kappa and lambda light chains IgA and IgM were also within the reference ranges although within their lower limits *N* = normal subject

Studies on urine

Light chains could not be detected in the urine after agarose gel electrophoresis of different samples concentrated 300 times Immunoelectrophoresis of the urine also failed to demonstrate Bence Jones protein Heller s and Bradshaw s tests for protein were negative

Studies on bone marrow

Light microscopy Bone marrow aspirate stained by May Grunwald Giemsa demonstrated the presence of 45% pathological cells which bore a distinct resemblance to plasma cells The nucleus in several cells was immature with a distinct nucleolus and a perinuclear halo was often recognized Inclusion bodies were also seen in some of the cells Histological examination of a bone marrow specimen demonstrated no signs suggesting metastatic infiltration

Electron microscopy These studies showed a large number of bone marrow cells with well preserved structure and with an appearance characteristic of plasma cells (Fig 2) In some cells the nuclei appeared immature with a prominent nucleolus The cytoplasm contained typical cisternae of granular endoplasmic reticulum (GER) containing an amorphous material The GER was markedly dilated in some cells The Golgi apparatus (GOL) was prominent and an abundance of structures with the appearance of smooth endoplasmic reticulum (SER) could be seen in many cells (Fig 3) These large tubules and vesicles also contained amorphous material with the electron density similar to that seen in GER Small inclusions sometimes with a myelin structure were seen outside the cisternae of the endoplasmic reticulum These inclusions varied in size and electron density but there was no evidence of crystalline structures

Immunofluorescent studies Large cellular infiltrations of the bone marrow were stained with anti F(ab)$_2$ Of the anti F(ab)$_2$ positive cells 85% were stained with anti IgG (Fig 4) and 90% with anti kappa light chain antisera 15% were stained with anti IgA 10% with anti IgM and 10% with anti lambda light chain antisera Anti IgD and anti IgE were not tested

Studies on peripheral blood

Light microscopy The peripheral blood contained 5 8×10^9 leucocytes per liter with a normal distribution as evaluated after differential count No rouleaux phenomenon was observed nor were any plasma cells obtained in the peripheral blood smear

Studies on the various lymphocyte populations

B lymphocytes 12% of the peripheral blood lymphocytes were positive with anti F(ab)$_2$ 7%



Fig 2 Electron m crograph of plasma cell from bone marrow with a nucleus (N) and nucleole (NU) Characterist c pattern of GER and a well developed GOL

with anti IgM 6% with anti IgD 1% with anti IgA 2% with anti IgG antiserum and less than 1% with anti IgE antiserum Furthermore 7 5% of the cells stained with anti kappa and 4% with anti lambda type light chain antisera

The cytocentrifuge preparations of the peripheral od lymphocytes did not stain with the anti ab) or the anti IgG antiserum

T lymphocytes 64% of the T lymphocytes formed E rosettes

EA rosette forming cells 20% of the cells demonstrated EA rosette formation

Functional lymphocyte studies

The unspecific mitogens PHA and PWM induced a definite DNA synthesis in the patient s lymphocytes These responses were of the same magnitude as in lymphocytes from a normal blood donor

Scintigraphic studies of the skeleton

In the dorsal view the uptake of the radioisotope was increased as compared to normal in several of the costae on both sides (Fig 5) in the sternoclavicular joint on the right side and in the left hip Generally the uptake of radioisotope in the skeleton was poor as compared to normal healthy subjects The lesions in the hip and sternum were also detected by conventional X ray determinations

DISCUSSION

Eighty five per cent of the plasma cells in the patient s bone marrow were stained with anti IgG and 90% with anti kappa light chains In other words the majority of the plasma cells seemed to belong to a single clone Since this intracellular immunoglobulin had normal antigenic propert es the molecules are probably structurally intact Since no M component was detected in the patient s plasma one or more defects might be present in the plasma cells which impede secretion or the synthesis of immunoglobulins

It seems that two major types of non secretory plasma cell dyscrasias exist one in which the synthesis of immunoglobulins in intact plasma cells takes place but in which secretion of paraprotein from the plasma cells is inhibited Ultrastructurally these plasma cells appear to be normal with a well developed ergastoplasma and other characteristics which are usually found in active protein synthetizing cells Normal immunoglobul ns are



Fig 3 Deta l from a plasma cell conta n ng GER and SER n the area close to the Golgi zone *INC* = nc us ons *MIT* m tochondria

usually suppressed n serum In the other major type the mmunofluorescent sta n ng of plasma cells fa ls to demonstrate intracellular immunoglobul ns no paraprote n is detected in serum and normal immunoglobul ns are severely depressed Ultrastructurally however these plasma cells can be normal or abnormal (16) In other words the defect m ght e ther be one of fa l ng secret on from the cells (non secretors) or one of fa l ng m munoglobul n synthes s (non producers)

Our pat ent d ffered somewhat from both these categories n that normal mmunoglobul n levels were found in serum immunoglobul ns were pres ent in the plasma cells as demonstrated by m munofluorescent techn que and the ultrastructure of many of the plasma cells was normal However it should be po nted out that the system of ves cles and c sternae called smooth endoplasmic ret culum may in fact represent GER where the ribosomes have become detached Th s rema ns however an

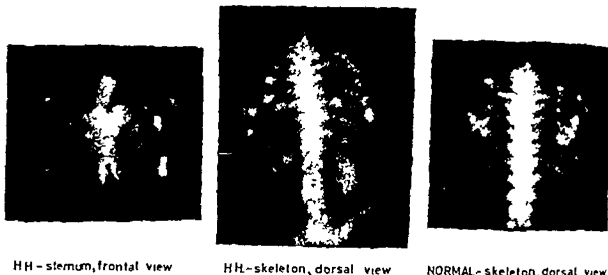


Fig 5 Scintigraphic studies of the patients skeleton (*H H*) Note the extensive lesions of increased radioactive uptake in sternum and ribs and compared to a normal skeleton Pictures taken with General Electric Maxicamera ^{99m}Tc labelled methylene disphosphonate

open question but the possibility exists that the defect observed may be related to structures of the endoplasmic reticulum

The observed inclusions appeared to be located outside the endoplasmic reticulum and are assumed to be phagosomes No paraprotein was detected in serum Extensive skeletal lesions were present which showed a positive uptake of ^{99m}Tc labelled methylene diphosphonate

Several possibilities are entertained to explain a k in the secretory process Lack of intracellular protein for the transport of light chains through the endoplasmic reticulum has been suggested (15) Attachment of carbohydrate has also been considered a prerequisite for release of immunoglobulin molecules (19) A block in this attachment could account for the secretory defect A faulty heavy and light chain synthesis prior to assembly has also been considered (1)

Few studies—with conflicting results—have been devoted to the investigation of different lymphocyte populations in patients with these types of plasma cell dyscrasias It is generally accepted that plasma cells develop from circulating B lymphocytes Subpopulations of T lymphocytes like helper T lymphocytes and suppressor T lymphocytes may influence this maturation (18) Our studies indicated the presence of a normal number of both B and T lymphocytes The lymphocytes also responded normally to stimulation with unspecific mitogens These results indicate that the defect in the immunoglobulin synthesis and/or secretion resides in the plasma cells themselves and not in the antecedent B lymphocytes

The immunofluorescent studies of the cytocentrifuge preparations of peripheral lymphocytes also indicated that no blast cells with intracellular immunoglobulins that could represent intermediate stages between the B lymphocytes and the malignant plasma cells were circulating in peripheral blood

Catovsky et al (4) reported on a normal lymphocyte response on the basis of the percentage of blast cells found and the incorporation of tritiated thymidine They recognized binucleated blast cells which probably represented an abnormal response

In contrast to our findings a diminished response to PHA was reported by Salmon and Fudenberg (23) and Campbell et al (3) Mellstedt and Holm (20) found that the stimulative effect of PWM on DNA synthesis was significantly decreased by treatment with melphalan and prednisone The stimulation by concanavalin A and PHA was not However our patient was hitherto untreated Normal T cell function as in our patient has been reported before in myelomatosis

Few clinical features differentiate secretory plasma cell dyscrasias from non secretory Severe reduction of normal serum immunoglobulins was the rule in the cases of non secretory

myelomatosis described in the literature (11 22 24) Almost normal amounts of IgG IgA and IgM were detected in our patient s serum Consequently the patient also exhibited normal bone marrow plasma cell populations This suggestion was verified in that 4% of the plasma cells were positively stained with anti IgA and 8% with anti IgM Probably it is just a question of time before these normal plasma cell populations will be destroyed by the malignant invasive clone

Median survival of patients with non secretory plasma cell dyscrasias is shorter than that of patients with regular myelomatosis (2) However a prolonged life span as compared to secretory myelomatosis has been observed in small series of patients (6 14 16) It is of interest that cases with non secretory plasma cell dyscrasias very rarely develop renal complications which are frequent and severe in secretory myelomatosis (21) probably due to excretion of free light chains

The differential diagnosis between malignant infiltration of the bone marrow and non secretory plasma cell dyscrasias with skeletal destructions might be difficult Combination of immunofluorescent techniques electron microscopy and conventional immunological techniques however will give the answer in most cases In elderly persons with skeletal involvement but without detectable paraprotein in serum the possibility of a non secretory plasma cell dyscrasia should be considered

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ANNOUNCEMENTS

A Symposium on Infarct Size will be held in Utrecht the Netherlands April 9–10 1979

Further information Professor Dr F L Meyler Department of Cardiology University Hospital Catharijnesingel 101 3500 Utrecht The Netherlands

The Sixth Biennial Program on Current Methods of Immunologic Research and Diagnosis will be offered by the Center for Immunology of the State University of New York at Buffalo June 4–22 1979

Further information James F Mohn M D The Center for Immunology State University of New York at Buffalo 210 Sherman Hall Buffalo New York 14214 USA

Deadline for applications March 31 1979

A Conference on Well Care Systems of the Future will be arranged by the International Health Evaluation Association and Salutis Unitas in Stockholm Sweden May 30–June 1 1979

Further information P Hall M D AMRAB Halsocentralen Sophiahemmet Box 5605 S 11486 Stockholm Sweden

International Course in Chest Radiology and other aspects of chest disease will be held in Stockholm Sweden June 11–13 1979 Organized by the Fleischner Society a non profit organization in memory of Felix Fleischner Harvard University Boston Interested colleagues are invited to participate

Information B Nordenstrom M D Professor of Radiology c/o Reso Congress Service S 10524 Stockholm Sweden

Eugen Werle Preis Die Boehringer Ingelheim Diagnostika GmbH hat den mit DM 10000 – dotierten Preis gestiftet Ziel des Preises ist die Forderung der Diagnostik auf dem Gebiet der klinischen Chemie und der klinischen Mikrobiologie Um den Eugen Werle Preis kann sich jeder Wissenschaftler mit einer methodischen Arbeit bewerben Zur Bewerbung zugelassen sind noch nicht veroffentlichte wie auch publizierte Arbeiten deren Veroffentlichung jedoch nicht vor der letzten Preisverleihung (15 11 77) liegen darf Die Arbeit muß zu einer wesentlich verbesserten diagnostischen Aussage fuhren

Fur die Verleihung 1979 soll die Arbeit in dreifacher Ausfertigung bis zum 31 03 79 an den Stiftungsrat z Hd Herrn Dr med H Berensmann Generalsekretär der Deutschen Gesellschaft zur Forderung der Medizinischen Diagnostik Jahnstr 32 Ärztehaus 7000 Stuttgart 70 eingesandt werden Das Manuskript selbst darf keinen Namen und keinen Hinweis auf den Autor enthalten Jede Arbeit ist mit einem Kennwort zu versehen In einem zusatzlichen verschlossenen Umschlag der das Kennwort der Arbeit tragen muß sind anzugeben Vor und Zuname Beruf Stellung und Tatigkeit Adresse sowie Kennwort der Arbeit

Acta Med Scand 204 445–451 1978

Non-Secretory or Low-Secretory Myeloma with Intracellular Kappa Chains

Report of Six Cases and Review of the Literature

Ingemar Turesson and Anders Grubb

From the Departments of Internal Medicine and Clinical Chemistry University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT This report concerns six cases of multiple myeloma characterized by either no demonstrable monoclonal immunoglobulin in plasma or urine or by trace amounts (≤0 1 g/l) of monoclonal kappa chains in the urine In all cases there was an infiltration of the bone marrow by plasma cells containing kappa chains but no heavy chains A retrospective analysis was made of 126 consecutive cases of Bence Jones myeloma The number of kappa and lambda cases was approximately the same All cases secreting ≤0 1 g light chains per l urine were of kappa type This contrasts with a kappa/lambda ratio of 1 4–1 9 among reported series of M components containing both heavy and light chains A review of reported cases of non secretory myeloma revealed a preserved capacity for Ig synthesis in the majority of cases and among these a preponderance of kappa chain producing clones These observations might be explained by a higher tendency for kappa chain producing cells to mutate to low secretors or to cells producing abnormal light chains which are catabolized rapidly The clinical data from our patients do not indicate a more pessimistic prognosis in non or low secretory myeloma than in other cases of multiple myeloma

Key words Non secretory myeloma Bence Jones protein myeloma cells intracellular immunoglobulins

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In about 1% of patients with multiple myeloma no monoclonal immunglobulin can be detected in plasma or urine (12) The term non secretory myeloma has been applied to this condition Conflicting reports exist regarding the natural history of non secretory myeloma indicating both a more and a less favourable prognosis (12 15) Immunofluorescence and immunoperoxidase studies of intracellular immunoglobulins in myeloma cells suggest that patients with non secretory myeloma can be subdivided into two categories those who do not synthesize and those who synthesize but do not secrete monoclonal immunoglobulin (2 3 5 6 9 18 20 21 22 23 25 26 28 29 31 34)

We report six cases of multiple myeloma characterized by no demonstrable monoclonal immunoglobulin in plasma Either no or only trace amounts of monoclonal light Ig chains were found in the urine All had a predominance of bone marrow cells containing kappa chains in their cytoplasm We draw attention to the preponderance of kappa chain producers among non secretory and low secretory myeloma cases

CASE REPORTS

Case 1

A man born in 1937 was referred to the Department of Medicine in Ljungby in March 1975 because of tiredness and headache He was found to have a mild hypertension proteinuria (1–2 g/l) and increased serum creatinine level (196 μmol/l) A plasma protein analysis showed pronounced hypogammaglobulinemia but agarose gel electrophoresis of plasma and concentrated urine failed to show any monoclonal immunoglobulin The bone marrow contained 15% plasma cells the majority having an immature nuclear chromatin structure and X ray examination revealed widespread lytic lesions in the skull and pelvis An immunofluorescence examination of the bone marrow was performed in April 1975 By this time immunoelectrophoresis of plasma and concentrated urine revealed small amounts of monoclonal kappa chains amounting in the urine to 0 016–0 055 g/l on repeated examinations The patient was treated with intermittent courses of melphalan and prednisone His general condition and renal function have improved and the number of bone marrow plasma cells decreased He is alive and in good health 33 months after the diagnosis

Case 2

A woman born in 1936 was referred to the Department of Orthopedic Surgery in Malmo in April 1976 because of



Fig 1 Bone marrow plasma cells from case 6 (*a*) Smear Pappenheim stain (*b*) Cytocentrifuge slide Anti kappa FITC Kodak Tri X Pan ×850

long standing lumbar pain which had started after a minor trauma in the autumn of 1975 X ray examination revealed lytic lesions in the lumbar and thoracic vertebral column and several ribs Serum creatinine level was 124 μmol/l A bone marrow smear contained 7% plasma cells with pronounced anaplastic features Plasma immunoglobulin levels were moderately depressed but no M-component could be detected in plasma or urine by agarose gel electrophoresis On repeated examinations immunoelectrophoresis revealed monoclonal kappa chains in the urine in a concentration of 0 040–0 100 g/l An immunofluorescence examination of the bone marrow was performed and daily treatment with melphalan was started There was a rapid decrease of pain and the patient s general condition greatly improved She is alive in good health 20 months after the diagnosis

Case 3

A man born in 1909 who suffered a myocardial infarction in June 1974 was referred three months later to the Department of Medicine in Trelleborg because of anemia (Hb 112 g/l) and high ESR (66 mm/h) Plasma immunoglobulin levels were depressed X ray examination revealed wide spread lytic lesions in the skull pelvis and proximal femora and a bone marrow smear contained 45% plasma cells many of which were atypical with prominent nucleoli Agarose gel electrophoresis of plasma and concentrated urine failed to demonstrate any monoclonal immunoglobulin The patient was treated with intermittent courses of melphalan and prednisone until Feb 1976 when he was referred for immunofluorescence examination of the bone marrow During this time repeated plasma and urine protein analyses and bone marrow examinations confirmed the cytological picture of myeloma and absence of M-component During the spring of 1976 there were clinical and radiological signs of progress of the disease and in July 1976 the patient was admitted to the hospital because of confusion rightsided central paralysis of the VIIth cranial nerve and hypercalcemia His condition deteriorated rapidly and he died within 7 days No autopsy was performed

Case 4

A woman born in 1907 fractured her right humerus in April 1974 She was referred to the Department of Medicine in Östersund X ray examination revealed numerous lytic lesions in both humeri and in the ribs Bone marrow smears from the sternum and the fractured humerus showed a massive infiltration of atypical plasma cells Plasma immunoglobulin levels were depressed There was no M-component in the plasma but small amounts of monoclonal kappa chains (0 029 g/l) in the urine Serum creatinine level was 70 μmol/l The patient was treated with melphalan and prednisone the fracture healed and she was free from symptoms for two years An immunofluorescence examination of the bone marrow was performed in Sept 1975 In the autumn of 1976 there were signs of progression with new lytic lesions and slight hypercalcemia but only trace amounts of Bence Jones protein in the urine Cytostatic treatment was reinstituted and the patient s condition improved again She is alive 44 months after the diagnosis with moderate back pain as her sole complaint

Case 5

A man born in 1910 was referred to the Department of Surgery in Malmö in July 1977 due to nausea and weight loss He was found to have a high ESR (102 mm/h) and increased levels of serum creatinine (4[illegible] μmol/l) and serum calcium (3 9 mmol/l) X ray examination revealed pronounced osteoporosis in the vertebrae and lytic lesions in the pelvis A bone marrow smear contained 50% plasma cells No M-component could be detected in the plasma but the urine contained monoclonal kappa chains in a concentration of 0 070 g/l An immunofluorescence examination of the bone marrow was performed and the patient was treated with sodium phosphate and intermittent courses of melphalan and prednisone The patient had a pathological fracture of his left femur but the condition slowly improved and he was able to leave the hospital in Sept 1977 with serum creatinine and calcium levels within normal limits *He is alive in fairly good health 5 months after the diagnosis*

Table I *Immunoglobulin containing bone marrow cells in six cases of multiple myeloma k/l ratio H/L ratio and number of kappa positive cells per 1 000 nucleated bone marrow cells*

Case no	k/l ratio	H/L ratio[b]	κ positive cells per 1 000 nucleated cells
1	99	<0 01	436
2	15	0 14	107
3	136	0 02	82
4	660	0 01	290
5	455	0 01	515
6	117	0 02	281
Polyclonal bone marrow[c]	1 5	1 1	9

No of κ positive/λ positive cells
[b] No of α μ γ δ or ε positive/κ or λ positive cells
Patients without plasma cell dyscrasia Figures from ref 32

Case 6

A woman born in 1904 started to complain of skeletal pain in the autumn of 1974 In Feb 1976 she was referred to the Department of Medicine in Eksjo A bone marrow smear contained 80% plasma cells and X ray examination revealed lytic lesions in the vertebral column and in the pelvis Serum creatinine level was 64 μmol/l There was severe depression of plasma Ig levels but no monoclonal immunoglobulin could be detected in plasma or urine The patient was treated with intermittent courses of melphalan and prednisone Her condition gradually improved the bone marrow plasma cell number decreased and in the autumn of 1977 she had only slight intermittent back pain Repeated analyses of plasma and urine failed to detect monoclonal Ig An immunofluorescence examination of the bone marrow was performed in Sept 1977

Dr R Bachmann Department of Medicine Trelleborg Dr S Olsson Department of Medicine Ostersund Dr G Korsman Department of Medicine Eksjo and Dr K A Svensson Department of Medicine Ljungby made the initial diagnosis of multiple myeloma in four cases referred the patients and thus made this study possible

METHODS

Bone marrow was aspirated from the sternum or the iliac crest A single cell suspension was prepared The cells were sedimented on cytocentrifuge slides fixed and stained by an immunofluorescence procedure for the detection of intracellular immunoglobulin using FITC (fluorescein isothiocyanate) labelled monospecific antisera against human immunoglobulin alpha mu gamma delta and epsilon heavy chains (Nordic Immunological Laboratories Tilburg The Netherlands) and kappa and lambda light chains (Dakopatts AS Copenhagen Denmark) The details of the technique have been reported earlier (11 32) The slides were examined in a Leitz Orthoplane fluorescence microscope positive cells enumerated and the following calculations made ratio of cells positive for kappa chains to cells positive for lambda chains (k/l ratio) ratio of cells positive for heavy chains to cells positive for light chains (H/L ratio) and the number of cells containing Ig chains per 1 000 nucleated bone marrow cells

Plasma and urine were collected before treatment and subjected to agarose gel electrophoresis (14) Plasma IgA IgM and IgG levels were determined by electroimmunoassay (8 16) Urinary light Ig chains were quantitated by single radial immunodiffusion of concentrated urine (19) The antisera against kappa and lambda chains were raised in rabbits by repeated s c injections of a pool of ten purified human kappa or lambda chains containing both monomers and dimers The antisera were rendered monospecific by appropriate absorption with glutaraldehyde insolubilized human proteins A pool of ten pure kappa or lambda chains different from the pools used for immunization was used as standard in the immunochemical quantitation These standard pools contained both monomers and dimers of the light Ig chains Immunoelectrophoresis was performed with antisera against alpha mu gamma kappa and lambda chains on plasma and concentrated urine These procedures had a detection limit for M components in plasma of about 0 1 g/l and in urine of about 0 1 mg/l

RESULTS

The results of the immunofluorescence examination of the bone marrow are presented in Table I In all cases there was a pronounced dominance of kappa positive cells over lambda positive cells and the vast majority of cells contained no heavy chains The number of kappa-containing cells was increased in all cases varying from 82 to 515 per 1 000 nucleated bone marrow cells The positive cells had a brilliant cytoplasmic fluorescence of the same type as seen in other myeloma cases (Fig 1) Staining for surface membrane bound immunoglobulins on the myeloma cells was performed in only one case Only kappa chains were demonstrated The myeloma cells displayed no consistent morphological features distinguishing them from other myeloma cells judged from Pappenheim stained smears

The results of the plasma and urine protein analyses are presented in Table II All patients had depressed plasma levels of at least one Ig class and most patients had low plasma levels of all Ig classes In four cases monoclonal kappa chains were detected in the urine in concentrations ranging from 0 020 to 0 100 g/l while in two cases the urinary concentration of monoclonal light chains was below

Table II *Plasma immunoglobulin levels and urinary light chain concentrations in six cases of low secretory myeloma*

Analyses were performed before cytostatic treatment unless otherwise indicated (n d = not determined) The sensitivity of the measuring procedure was increased in these cases by using highly concentrated urinary samples

Case no	Plasma Ig concentration (g/l)			Urinary light chain concentration (g/l)		Immunoelectrophoresis of concentrated urine
	IgA	IgM	IgG	κ	λ	
1	0 2	0 1	1 5	0 050	<0 010	Abnormal kappa arc
2	0 4	0 4	9	0 100	<0 010	Abnormal kappa arc
3	0 3	n d	5 5	<0 010	<0 010	No monoclonal Ig
4	0 13	0 3	6	0 029	<0 010	Abnormal kappa arc
5	0 02	0 02	3	0 020	<0 010	Abnormal kappa arc
6	0 4	0 2	4	<0 010	<0 010	No monoclonal Ig
Healthy individuals	0 5–3 0	0 4–2 0	7–15	<0 010	<0 010	

0 0001 g/l Immunoelectrophoresis of urine in these *two cases was performed after treatment when the* number of plasma cells in the bone marrow was still high No monoclonal Bence Jones protein was detected

A retrospective analysis was made of 2 500 cases of monoclonal gammapathy registered at the Department of Clinical Chemistry in Malmo from June 1969 to September 1977 All cases with monoclonal light chains in plasma and/or urine but no complete plasma monoclonal immunoglobulin were collected and grouped according to clinical diagnosis and light chain type (Table III) 126 cases were classified as myeloma and 8 cases were considered non aluable owing to insufficient data The numbers cases with kappa and lambda chains were approximately equal The urinary concentration of light chains before treatment was available in 103 cases of multiple myeloma (Fig 2) All cases with Bence Jones protein concentration below 0 1 g/l were of the kappa type (these include the cases presented in this report) The urine volume was not known and the 24 hour urinary output of light chains could thus not be calculated Plasma samples taken at the time of diagnosis were available from 102 cases of Bence Jones myeloma There was no significant difference in the frequency of increased serum creatinine levels between kappa and lambda cases (Table IV)

DISCUSSION

All six patients in the present series had clinical signs of multiple myeloma with widespread osteolytic lesions and an increased number of bone marrow plasma cells In four cases trace amounts *of monoclonal kappa chains were detected in* the urine while two cases may be regarded as non secretory myeloma The immunofluorescence studies indicated a preserved capacity for kappa chain synthesis even in the non secretory cases and confirmed the monoclonal nature of the plasma cell proliferation Earlier investigations of non-secretory myeloma have demonstrated intracellular Ig in 37 of 44 cases examined with immunofluorescence or immunoperoxidase techniques (2 3 5 6 9 18 20 21 22 23 25 26 28 29 31 34) The true nature of the phenomenon of non secretion in these cases is not clear Biosynthetic studies in some cases have shown either no secretion of Ig (20) in vitro or secretion of very small quantities of Ig with a carbohydrate composition different from that of the intracellular Ig (18) The

Table III *All cases of plasma cell dyscrasia secreting immunoglobulin light chains only registered 1969–77 in Malmö grouped according to clinical diagnosis and light chain type*

Clinical diagnosis	No of cases		
	Total	κ	λ
Multiple myeloma	126	61	65
Chronic lymphocytic leukemia	4	3	1
Malignant lymphoma	2	2	0
Benign monoclonal gammapathy	2	3	1
Primary generalized amyloidosis	6	3	1
Sarcoidosis	1	0	1
Chronic glomerulonephritis	1	1	0
Not classified	8	4	4
Total	150	76	74

Table IV *Serum creatinine concentrations in 102 cases of Bence Jones myeloma*

	κ		λ	
Serum creatinine (μmol/l)	No	Per cent of all kappa cases	No	Per cent of all lambda cases
≥400	6	12	9	17
300–400	10	20	13	25
200–300	19	39	16	30
<200	14	29	15	28
Total	49		53	

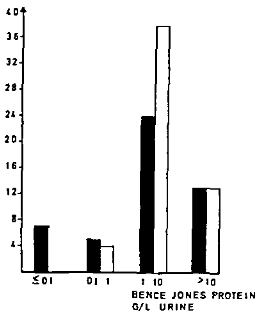


Fig 2 Urinary concentration (g/l) of kappa (■) and lambda (□) chains in 103 cases of Bence Jones myelomas

finding of intermittent urinary output of minute quantities of Bence Jones protein as well as amyloidosis in other series of non secretory myeloma (23) favors the hypothesis that many cases are not truely non secretory and that no sharp limit exists against low secretory myeloma If some secretion does occur the low output may be due to unusually rapid catabolism of possibly abnormal Ig chains Such a mechanism has been claimed in mouse myeloma (4) The kappa chains of one of the patients in the present series were purified and subjected to SDS-polyacrylamide electrophoresis

Their molecular size did not differ from that of kappa chains from other Bence Jones myeloma cases The simple explanation that the low urinary output of Bence Jones protein in the present series depends on low tumor cell mass seems unlikely in view of the widespread osteolytic lesions and large number of bone marrow plasma cells

The finding of kappa chains in six consecutive cases of non or low secretory myeloma prompted an investigation of the relative frequency of kappa and lambda chains in myeloma and other monoclonal gammapathies In the largest reported series of M components containing heavy and light chain there is a preponderance of the kappa type with a ratio of 1 4 to 1 9 (Table V ref 7 17 24 33) This corresponds well with the ratio 1 5 of kappa to lambda containing cells in normal human bone marrow (10 32) and fits well with the hypothesis that the risk of neoplastic transformation is the same for all Ig producing cells irrespective of light chain type In cases with only Bence Jones protein the numbers of kappa and lambda types are however equal (Table VI ref 1 27) Nontheless all myeloma cases in the present series with low secretion rates (urinary light chain concentration ≤0 1 g/l) were of the kappa type ($p<0.05$)

Table V *M-component light chain type in patients with M components containing heavy and light immunoglobulin chains*

Reference	Heavy chain	No of cases Total	κ	λ	κ/λ ratio
Laurell (16)	A G D	470	284	186	1 5
Pruzanski and Ogryzlo (24)	A M G D	289	170	119	1 4
Galton and Peto (7)	A G D	210	130	80	1 6
Waldenstrom (33)	A M G D	853	559	294	1 9

Myeloma cases only

Table VI *M-component light chain type in patients secreting light immunoglobulin chains only*

Reference	No of cases Total	κ	λ	κ/λ ratio
Alexanian et al (1)	80	42	38	1 1
Shustik et al (27)	97	52	45	1 2
Malmo 1969–77	150	76	74	1 0
Malmo 1969–77 myeloma cases only	126	61	65	0 9

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Quantitation of J Chain in Human Biological Fluids by a Simple Immunochemical Procedure

Anders O Grubb

From the Department of Clinical Chemistry University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT The molecular form and immunochemical properties of the J chain populations released on reduction and carboxymethylation of normal human plasma, milk saliva and of plasma containing IgA or IgM M components were investigated A procedure was devised to release the entire J chain population from these various sources and to produce immunochemically identical J chain populations containing only J chain monomers An identical standard J chain population was purified and quantitated by physiochemical means A specific rabbit anti J chain antiserum was raised against this pure J chain population A simple and rapid immunochemical method for J chain quantitation in complex biological fluids as well as in solutions of pure polymeric immunoglobulins was constructed on these grounds The J chain concentration was found to be (mean ±S D) 1 74±0 65 μM in normal human plasma 1 94±1 21 μM in human milk and 0 48±0 26 μM in human saliva The J chain/IgA molar ratio was found to be (mean±S D) 0 45±0 07 in human milk and 0 52±0 09 in human saliva when the IgA concentration was expressed as monomeric units per volume unit The range of the J chain/IgA molar ratios in plasma samples with highly concentrated IgA M components was 0–0 64 The J chain/IgM molar ratio in plasma samples with highly concentrated IgM M-components was between 1 and 2 when the IgM concentration was expressed as pentameric units per volume unit

Key words J chain immunochemistry quantitation
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A small polypeptide chain demonstrated to occur in some polymeric immunoglobulins (7 8 28) was shown in 1970 by Halpern and Koshland (11) to be a novel component of human and rabbit polymeric IgA The authors named this new immunoglobulin part the J chain since they considered that its function was to assist in the joining of the monomeric immunoglobulin units into polymeric molecules Mestecky et al (24) demonstrated in 1971 the J chain to be a covalently bound part of human pentameric IgM Subsequent studies have shown it to be present in the macroimmunoglobulins of a number of vertebrate species (17) In addition to its role in the joining of monomeric IgA and IgM units the J chain seems to be required for the combination of the polymers with secretory component (5) J chain synthesis has been demonstrated not only in plasma cells secreting polymeric IgA and IgM but also in IgG containing lymphoid cells (2 15) It has been speculated that J chain plays an important role in the regulation of the Ig synthesis by various antigen stimulated lymphoid cells (4 17 23) Progress in the elucidation of the part played by the J chain in the antibody response has however been delayed by lack of a simple and rapid method for quantitation of J chain in complex biological fluids Presently available physicochemical methods (9 12 25) all require a purification step and are therefore troublesome and sensitive to variations in recovery No values for the J chain concentration in human plasma milk or saliva have been reported

The present work describes a simple rapid and sensitive immunochemical procedure for J chain quantitation in biological fluids The method is based upon a complete release of J chains in monomeric form by reduction and alkylation of the biological fluid studied A subsequent electroimmunoassay of the J chain population is performed using an antiserum against reduced and alkylated

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Abbreviations SDS=sodium dodecyl sulfate barbital EDTA buffer=0 075 M barbital buffer pH 8 6 containing 2 mM ethylenediaminetetraacetic acid DTT=dithiothreitol Tris buffer=2 0 M tris-(hydroxymethyl) amino-methane buffer pH 8 6

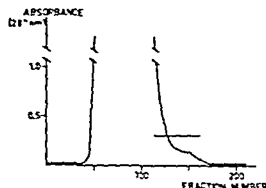


Fig. 1. Sephadex G 200 Superfine chromatography in 0.1 M ammonium bicarbonate at +6 C of a reduced and alkylated IgM M-component. 3-ml fractions were collected at a flow rate of 3 ml/h in a ... cm column. The J chain-rich fractions denoted by the horizontal line were pooled and lyophilized.

J chain and a J chain standard population of monomeric reduced and alkylated molecules. The work also reports the J chain concentration as obtained by this method in normal human plasma, milk and saliva as well as in plasma samples with highly concentrated IgM or IgA M-components.

MATERIALS

Agarose with low electroendosmosis was purchased from L'Industrie Biologique Française S. A., Gennevilliers, France and from Marine Colloids Inc., Rockland, Maine, USA. Sephadex G 200 and Dextran T 10 from Pharmacia Fine Chemicals, Uppsala, Sweden. Ultrogel AcA 54 from LKB-produkter AB, Bromma, Sweden. DEAE-Cellulose from Whatman Ltd, Springfield Mill, England. guanidinium chloride and Coomassie Brilliant Blue R 250 from Schwarz/Mann, Orangeburg, N.Y., USA, and complete Freund's adjuvant from Difco Laboratories, Detroit, Mich., USA. All other chemicals were of reagent grade and obtained from British Drug Houses Ltd, Poole, England. Monospecific rabbit antisera against human polyclonal IgM, IgA and against IgG Fc-fragments were available at our laboratory.

Fresh samples of human plasma and saliva were obtained from healthy laboratory personnel and fresh samples of milk from lactating healthy females. Plasma samples with highly concentrated IgM, IgG, IgD or IgA M-components were selected from samples sent to the Clinical Laboratory for Ig measurements. These samples were stored frozen at −20°C for up to a year before their J chain concentrations were determined.

METHODS

Electrophoretic procedures. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed as described by Weber and Osborn (33) and analytical and preparative agarose gel electrophoresis as reported by Johansson (14).

Immunochemical procedures. Single radial immunodiffusion was performed according to Mancini et al. (22), double radial immunodiffusion according to Ouchterlony (27), microimmunoelectrophoresis as described by Scheidegger (30) and crossed immunoelectrophoresis as described by Laurell (19) and Ganrot (10). 10% Dextran T 10 was incorporated in the antiserum-containing gel to increase the precipitation of antigen-antibody complexes (13). Laurell's procedure for electroimmunoassay (20) was slightly altered as described in Results.

Amino acid analysis. Quantitative amino acid analyses were carried out essentially according to Spackman et al. (31). Protein samples of about 1 mg were hydrolyzed in 6 M HCl at 110°C for 24 and 72 hours in sealed, evacuated Pyrex tubes. The hydrolyzates were analysed with the two-column system on a Jeol model JLC-5 AH automatic amino acid analyzer with the Jeol resin RC 1.

Purification of 19S and 8S IgM M-components. One 8S and two 19S IgM M-components were purified in large amounts from plasma samples from two patients with macroglobulinemia. One of the samples contained a highly concentrated 19S IgM(κ) M-component in addition to an 8S IgM(κ) M-component of lower concentration. The other sample contained a single concentrated 19S IgM(κ) M-component. The 19S M-components were purified by ammonium sulfate precipitation followed by repeated euglobulin precipitation and gel chromatography on Sephadex G 200. The 8S IgM M-component was purified by stepwise ammonium sulfate precipitation, dialysis of the appropriate fraction against phosphate buffer and ion exchange chromatography of the fraction on a DEAE cellulose column in the same buffer with elution of the M-component by a linear salt gradient. The M-component solution was then passed through a glutaraldehyde-

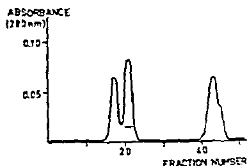


Fig. 2. Ultrogel AcA 54 chromatography in 0.05 M ammonium bicarbonate at +6 C of a J chain fraction partly purified by Sephadex G 200 chromatography and preparative agarose gel electrophoresis. Ten ml fractions were collected at a flow rate of 30 ml/h in a 9× 2.5-cm column. The fractions denoted by a horizontal line consisted of pure monomeric J chains, while the preceding fractions contained J chain dimers and polymers. Light absorbing buffer salts from the preparative gel electrophoresis were eluted in fractions 41–46.



Fig. 3. Analytical agarose gel electrophoresis in 0.075 M barbital buffer, pH 8.6, with 2 mM EDTA of (from left to right) normal human serum, a fraction containing monomeric J chains from the Ultrogel chromatography described in Fig. 2, a fraction containing polymeric J chains from the Ultrogel chromatography described in Fig. 2, the monomeric J chain population used as standard in the quantitative immunochemical procedure, a reduced and carboxymethylated 19S IgM M-component.

insolubilized (1) immunosorbent against IgG and IgA and finally gel chromatographed on Sephadex G 200. Analytical agarose gel electrophoresis, SDS-polyacrylamide gel electrophoresis and microimmunoelectrophoresis with use of an antiserum against human plasma failed to reveal any contamination of the purified M components. These were extensively dialyzed against distilled water and then lyophilized.

RESULTS

Production of pure reduced and carboxymethylated J chain for immunization

Of a pure IgM(κ) M component, 8.0 g were dissolved/suspended in 200 ml 0.075 M barbital buffer, pH 8.6, with 2 mM EDTA (barbital EDTA buffer). To the turbid solution was added 8 ml of the barbital EDTA buffer containing 250 mg dithiothreitol (DTT). After 2 hours in room temperature, during which period the solution became clear, 16 ml of a 2.0 M Tris HCl buffer, pH 8.6 (Tris buffer) containing 630 mg iodoacetic acid was added. After another 15 min in room temperature the mixture was chromatographed on a column of Sephadex G 200 Superfine (Fig. 1). The eluted fractions were monitored for their J chain content by analytical agarose gel electrophoresis (Fig. 3) and J chain rich fractions were pooled and lyophilized. The lyophilized substance was then dissolved in a small amount of barbital EDTA buffer and subjected to preparative agarose gel electrophoresis. Gel strips containing J chain were then cut out, frozen and thawed three times. Thereafter they were centrifuged to elute the J chain solution from the gel residue. This J chain solution was lyophilized, the substance dissolved in 0.05 M ammonium bicarbonate and chromatographed on a column of Ultrogel AcA 54 (Fig. 2). Two protein containing peaks were obtained, one consisting of monomers and the other of dimers and higher polymers of J chain (Fig. 3). The monomeric J chain fractions were pooled and lyophilized. SDS polyacrylamide gel electrophoresis, analytical agarose gel electrophoresis and microimmunoelectrophoresis were used to demonstrate the absence of contaminating proteins in this J chain preparation.

Production of an antiserum against reduced and carboxymethylated J chain and testing of its specificity

Of the lyophilized, reduced and carboxymethylated monomeric J chain preparation, 2 mg were dissolved in 1 ml 0.15 M NaCl. This solution was emulsified with 2 ml complete Freund's adjuvant and injected in the back feet and at some subcutaneous sites on the backs of three rabbits. Similar booster injections were given 2, 7 and 11 weeks after the primary immunization. The rabbits were then bled every two weeks. The specificity of the antisera was tested by crossed immunoelectrophoresis with up to 7% antiserum in the gel and with native human normal plasma or with reduced and carboxymethylated normal or agammaglobulinemic human plasma in the first electrophoretic step. Two of the three rabbits produced antisera which were considered monospecific since they only formed one precipitation peak with reduced and carboxymethylated normal human plasma but no precipitation at all with native normal plasma or with reduced and carboxymethylated

Table 1 *Amino acid composition of the monomeric standard J chain population*

Constituent	Found*	Results of Mole et al (26)
Aspartic acid	17 3	21
Threonine*	13 6	11
Serine*	10 3	8
Glutamic acid	13 9	15 5
Proline	8 1	8
Glycine	3 7	2
Alanine	6 5	6
Carboxymethylcysteine	6 6	8
Cystine	0	
Valine†	9 7	11
Methionine	1 8	1
Isoleucine	8 1	8
Leucine	8	8
Tyrosine	5 2	5
Phenylalanine	1 6	1
Lysine	5 5	5 5
Histidine	1 4	1
Arginine	8 3	9

Calculated on the basis of 8 residues of leucine per molecule *Values obtained by extrapolation to zero hours hydrolysis † Seventy two-hour hydrolysis value only

agammaglobulinemic human plasma After 3 bleedings without booster injections a significant decrease in the antiserum titer was noted

Production of a purified reduced carboxymethylated and monomeric J chain population for use as standard

A pure 19S IgM(κ) M-component was reduced carboxymethylated and fractionated on Sephadex G 200 exactly as described above for the production of J chain for immunization J chain rich fractions were identified by analytical agarose gel electrophoresis pooled and concentrated by pressure ultrafiltration at +4°C The concentrated J chain solution was then chromatographed on a column of Ultrogel AcA 54 under the conditions described in Fig 2 and the eluate monitored by agarose gel electrophoresis The fractions containing J chain were pooled concentrated by pressure ultrafiltration and rechromatographed on the Ultrogel column A single protein peak containing monomeric highly purified J chain was obtained Appropriate fractions were pooled concentrated by pressure ultrafiltration and divided into small aliquots which were frozen and stored at −24°C until used Analytical agarose (Fig 3) and SDS polyacrylamide gel electrophoresis demonstrated that this monomeric standard J chain population although not as pure as the J chain population used for immunization consisted to at least 90% of reduced and carboxymethylated J chain molecules

Molar concentration of J chain in the standard solution

The J chain concentration in the J chain standard solution was measured by three different procedures Firstly a measured volume of the solution was carefully lyophilized at high vacuum and its protein content was weighed thereafter Secondly the light absorption of the solution at 278 nm was used to calculate its J chain concentration from the extinction coefficient for J chain ($E^{1\%}_{1cm}$=7 0) given by Wilde and Koshland (34) Thirdly measured volumes of the standard solution were lyophilized their protein contents hydrolyzed in 6 M HCl for 24 and 72 hours and their total amino acid content determined The molar concentration of the various amino acids (excluding amino acids of low concentrations) were then used to calculate the molar J chain concentration in the standard solution by comparison with the total amino acid composition of J chain given by Mole et al (26) The results obtained by the three procedures were 207 191 and 167 μM respectively when a J chain molecular weight of 16422 daltons was assumed (26) The

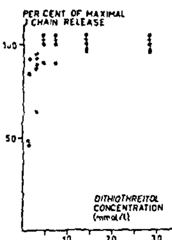


Fig 4 Influence of increasing dithiothreitol concentration on the J chain release from normal human plasma (○) milk (▲) saliva (□) a plasma sample with a J chain rich IgA M-component (●) and from a plasma sample with a 19S IgM M-component (+)

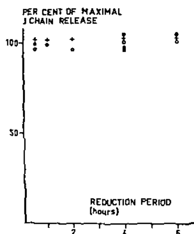


Fig 5 Influence of increasing reduction periods on the J chain release from normal human plasma (O) milk (▲) saliva (□) a plasma sample with a J chain rich IgA M-component (●) and from a plasma sample with a 19S IgM M-component (+) at a final dithiothreitol concentration of 7 mM

amino acid composition of the standard J chain population is given in Table I No trace of cystine could be seen in the chromatograms

Release of J chain from various biological fluids on reduction and carboxymethylation

Normal human plasma serum saliva and milk were used in these experiments as well as a plasma sample with a highly concentrated 19S IgM M component a plasma sample with a highly concentrated J chain rich IgA M component and a solution in barbital EDTA buffer of a pure 8S IgM M-component (10 mg/ml) Four parts of barbital EDTA buffer were added to one part of these solutions and thereafter increasing amounts of DTT in a constant small volume of barbital EDTA buffer After 2 hours in room temperature the solutions were mixed with Tris buffer containing a molar amount of iodoacetic acid 2 1 times the amount of DTT used for reduction After another 15 min in room temperature the J chain release in the samples was determined by the electroimmunoassay described further on The results are shown in Fig 4 As can be seen no further J chain release from the different biological fluids was observed by increasing the final DTT concentration beyond 7 mM No J chain release from the pure 8S IgM was observed at any DTT concentration

Table II *Release of J chain on reduction and carboxymethylation of different biological fluids in 0 075 M barbital buffer pH 8 6 with 2 mM EDTA compared to the release on reduction and carboxymethylation in 2 M Tris HCl buffer pH 8 6 containing 6 M guanidinium chloride*

Biological fluid	Release (%)
Normal human serum	92
Human milk	99
Human saliva	117
Plasma sample with a concentrated J chain rich IgA M-component	100
Plasma sample with a concentrated 19S IgM M-component	109



Fig 6 Analytical agarose gel electrophoresis in 0 075 M barbital buffer pH 8 6 with 2 mM EDTA of (from left to right) a 19S IgM M-component reduced and carboxymethylated in 0 075 M barbital buffer pH 8 6 the same 19S IgM M-component reduced and carboxymethylated in 2 M Tris HCl buffer pH 8 6 containing 6 M guanidinium chloride normal human plasma Before the electrophoresis the guanidinium chloride-containing solution was dialyzed against barbital buffer and cleared by centrifugation

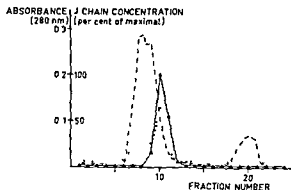


Fig 7 Ultrogel AcA 54 chromatography in 0 05 M ammonium bicarbonate at +6 C of reduced and carboxymethylated normal human plasma and of a marker protein solution Two ml fractions were collected at a flow rate of 6 ml/h in a 56 × 1-cm column The J chain concentration in the fractions was determined by electroimmunoassay ---=280-nm absorption of reduced and carboxymethylated plasma ▲—▲=J chain concentration =280 nm absorption of the marker protein solution containing horse apoferritin monomeric light Ig chains cytochrome C and Kunitz s trypsin inhibitor

Four parts of barbital EDTA buffer were added to one part of the biological fluids described above (excluding the 8S IgM solution) and then a small volume of a DTT solution giving a final DTT concentration of 7 mM After increasing periods of time the reduction was terminated by addition of Tris buffer containing iodoacetic acid After another 15 min at room temperature the J chain release was assessed by the electroimmunoassay Fig 5 gives the results After a reduction period of 2 hours no further release of J chain from the samples occurred

To one part of each of the biological fluids described above was added five parts of a solution of 6 M guanidinium chloride in Tris buffer Thereafter DTT in a small volume of Tris buffer was added to a final concentration of 7 mM and after 2 hours at room temperature the reduction was terminated by addition of Tris buffer containing iodoacetic acid All solutions were then extensively dialyzed at +4°C in barbital EDTA buffer The volumes of the solutions after the dialysis were recorded In parallel experiments the biological fluids were reduced and carboxymethylated as described above but with the guanidinium containing Tris buffer replaced by barbital EDTA buffer The J chain release from the biological fluids on reduction and alkylation under denaturing and non denaturing conditions was then

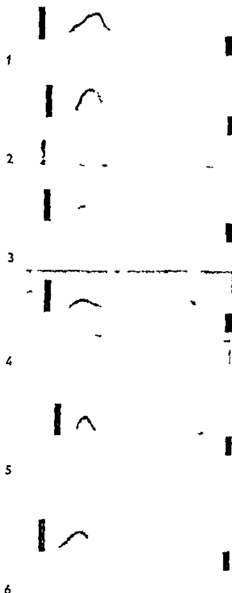
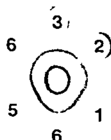


Fig 8 Crossed immunoelectrophoresis with the use of anti J chain antiserum of various reduced and carboxymethylated biological fluids *1*=the standard J chain solution *2*=normal human plasma *3*=milk *4*=saliva *5*=a plasma sample with a J chain rich IgA M-component *6*=a plasma sample with a 19S IgM M-component The long vertical lines denote the migration of an albumin marker in the first electrophoretic runs and the short verticals the application slots of the first electrophoretic runs

determined by electroimmunoassay Table II demonstrates that the same amount of J chain seems to be released under the denaturing and the non



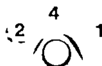


Fig 9 Double radial immunodiffusion of various reduced and carboxymethylated biological fluids The central wells contain an anti J chain antiserum *1* = standard J chain solution *2* = normal human plasma *3* = milk *4* = saliva *5* = a plasma sample with a J chain rich IgA M-component *6* = a plasma sample with a 19S IgM M-component

denaturing conditions Agarose gel electrophoresis of a solution of a pure 19S IgM M component reduced under both denaturing and non denaturing conditions gave the patterns depicted in Fig 6

Physicochemical and immunochemical properties of the standard J chain population and the J chain populations released from different biological fluids on reduction and carboxymethylation

One part of normal human plasma saliva and milk as well as of a plasma sample containing a concentrated 19S IgM M component and of a plasma sample containing a concentrated J chain rich IgA M-component was mixed with four parts of barbital EDTA buffer and reduced with DTT at a final concentration of 7 mM for 2 hours at room temperature The reduction was stopped by addition of Tris buffer containing iodoacetic acid 500 μl of these solutions and of the standard J chain solution were then gel chromatographed in sequential runs on a column of Ultrogel AcA 54 in 0 05 M ammonium bicarbonate A small amount of Kunitz s trypsin inhibitor was dissolved in the solutions before each run to mark the separation volume of the column Two-ml fractions were collected and their J chain concentration determined by electroimmunoassay The separation characteristics of the column were repeatedly monitored by running 500 μl of a solution containing horse

Fig 10 Electroimmunoassay of J chain in different human plasma samples The horizontal line on the cathodal side of the application holes s the pre dilutions of the J chain s solutio

Table III *J chain concentration in various biological fluids*

Biological fluid	No of samples	J chain (μM)[a]	IgA (μM)[b]	IgM (μM)[c]
Normal human plasma	18 (8 ♂ 10 ♀ Swedish Caucasians)	Mean 1 74 Range 0.57–2 61 S D 0 65	Mean 11 76 Range 4 08–30 47 S D 6 75	Mean 0 87 Range 0 38–1.51 S D 0 37
Plasma pool	From 1 000 registered blood donors	1 88	13 4	0 80
Normal human milk[d]	15 (from different Swedish Caucasians after varying post partum periods)	Mean 1 94 Range 0 68–4 36 S D 1 21	Mean 4 30 Range 1.38–9 27 S D 2 32	Range <0 02–0 05
Normal human saliva	16 (6 ♂ 10 ♀ Swedish Caucasians)	Mean 0 48 Range 0 15–0 95 S D 0 26	Mean 0 93 Range 0 29–1 76 S D 0 49	Range <0 01
Plasma samples with concentrated IgA M-components	92	Mean 50 2 Range <0 06–268 S D 70 0	Mean 280 Range 57 0–1 237 S D 203	Mean 0 16 Range 0 03–1 0 S D 0 14
Plasma samples with concentrated IgM M-components	33	Mean 35 5 Range 17 4–74 3 S D 14 3	Mean 4 3 Range <0 8–10 4 S D 2 2	Mean 20 6 Range 9 6–47 0 S D 9 6

[a] Calculated from a molecular weight of 16 422 (26)
[b] Calculated as 7S monomers with a molecular weight of 160 000
[c] Calculated as 19S pentamers with a molecular weight of 900 000
[d] The determinations were performed after removing the milk fat comprising 5–10 % (v/v) by use of a table centrifuge

apoferritin monomeric free light Ig chains cytochrome C and Kunitz s trypsin inhibitor on the column The J chain populations released from all tested biological fluids were eluted in one peak at the same volume as the standard J chain population and close to the elution volume of the free light Ig chains (Fig 7)

Crossed immunoelectrophoresis of the reduced and carboxymethylated biological fluids and of the standard J chain population with the use of an anti J chain antiserum gave similar or identical precipitation patterns for all solutions (Fig 8) Double radial immunodiffusion of the fluids using the same antiserum demonstrated a reaction of immunological identity between the standard J chain population and the J chain populations released from all the investigated fluids (Fig 9)

The immunochemical method for J chain quantitation in complex biological fluids

From the results described above the following procedure for immunochemical J chain quantitation in biological fluids was constructed One part of the biological fluid is mixed with four parts of 0 075 M barbital buffer pH 8 6 with 2 mM EDTA A small volume of a DTT solution is added so that the final DTT concentration becomes 7 mM After 2 hours at room temperature the reduction is terminated by addition of a small volume of a iodoacetic acid solution buffered to pH 8 6 and containing a molar amount of iodoacetic acid 2 1 times the added amount of DTT After a period of at least 15 min at room temperature the J chain concentration of the solution is determined by an electroimmunoassay system with the use of antiserum against reduced and carboxymethylated J chain in the agarose gel in 0 075 M barbital buffer pH 8 6 with 2 mM EDTA and 10 % Dextran T 10 The electrophoresis is run at 5 V/cm for 16 hours The J chain standard should

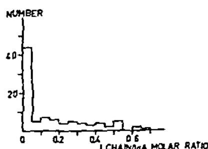


Fig 11 J chain/IgA molar ratio in 92 plasma samples with highly concentrated IgA M-components IgA concentration is expressed in monomeric units per volume unit

hain/IgA lar ratio	J chain/IgM molar ratio
an 0 45 nge 0 32–0.56) 0 07	
an 0 52 nge 0 35–0 78) 0 09 e Fig 11	
	Mean 1 8 *Range 1 0–2 4* S D 0 38

be a newly thawed solution of a pure monomeric reduced and carboxymethylated J chain population To have access to a large amount of a stable J chain standard it is also possible to use a J chain rich human plasma (e g plasma with a concentrated 19S IgM M-component) as J chain standard provided that its J chain concentration has been carefully determined by means of the procedure described above and with the use of a primary standard solution with a pure monomeric J chain population This secondary J chain standard should be reduced and carboxymethylated exactly as the samples with unknown J chain concentration at each determination An example of the precipitation pattern obtained on electroimmunoassay of the J chain concentration in various plasma samples is given in Fig 10

The sensitivity of this procedure was investigated by using decreasing amounts of antiserum in the gel in parallel with increasing dilutions of the J chain samples At J chain concentrations below 6 nM (=0 1 μg/ml) the precipitates became either too faint to be discernable or too low to be read with an acceptable precision

The precision of the method was tested by duplicate determinations of the J chain concentration in samples with both high and low J chain concentrations A coefficient of variation of about 5% of the mean for both high and low concentration values was obtained

The J chain concentration in various biological fluids

The J chain concentration in normal human plasma saliva and milk as well as in human plasma samples with highly concentrated IgM or IgA M components was determined by the immunochemical procedure described in this work Single radial immunodiffusion of the reduced and carboxymethylated samples was used to determine their concentrations of IgA and IgM The standard solution used for the immunoglobulin quantitations was reduced and carboxymethylated exactly as the test samples It was composed of a pool of serum from registered blood donors The immunoglobulin concentrations of this pool had been calibrated against the WHO international reference preparation for human serum IgG IgA and IgM (29) The anti IgA and anti IgM antisera had been raised in rabbits against polyclonal IgA and IgM populations

The results of these measurements are given in Table III and Fig 11

When the J chain concentrations in two plasma samples containing concentrated IgD M components and in about 200 plasma samples containing IgG M-components were determined concentration values equal to or below those in normal human plasma were obtained The J chain concentration in a concentrated solution of a purified 8S IgM M component was found to be below the sensitivity limit of the immunochemical method

DISCUSSION

The construction of an accurate immunochemical method for the quantitation of an antigen requires the consideration of three sets of problems The first concerns problems bearing on the physicochemical difference if any between the molecular population of the antigen to be determined in the test sample and that in the standard sample The second concerns problems related to the type of antiserum and the third problems related to the method used for comparison of the reactions between the antiserum and the test sample and between the antiserum and the standard sample If the

molecular populations in the test samples and in the standard sample are physicochemically identical the only requirement for the antiserum is that it must be monospecific and no specific demands are required for the method to compare the reactions between the antiserum and the molecular populations in the standard and test samples On the other hand if the molecular populations of the antigen in the standard sample and in the various test samples all differ from each other in physicochemical properties the construction of an immunochemical method for quantitation of this antigen with a high accuracy may be extremely difficult However this may still be achieved if the differences between the molecular populations are reduced as much as possible by physicochemical treatment of all samples and if in addition one selects an antiserum and a procedure for the antigen-antibody reaction which recognize as few as possible of the remaining differences

A method for immunochemical J chain quantitation has recently been proposed by Brandtzaeg (3) In this procedure the standard sample contained a heterogeneous J chain population differing considerably in physicochemical properties from the J chain populations in the test samples The evidence given for a complete J chain release from polymeric immunoglobulins was incomplete and the results varied considerably from experiment to experiment The procedure was only used to measure the chain content of purified polymeric immunoglobulins The results contradicted those obtained by others by physicochemical investigations of purified immunoglobulins

In the present work it proved possible by reduction and alkylation of all tested complex biological fluids to release J chain populations which had very similar or identical charge properties and which also gave reactions of immunochemical identity when tested with an unabsorbed monospecific rabbit antiserum raised against a pure reduced and alkylated J chain preparation In addition the released J chain populations seemed to be composed of only monomeric molecular species since they all eluted as homogeneous peaks on gel filtration and at the same volume as monomeric light Ig chains which are known to coelute with monomeric J chain populations (34) On these grounds it seems probable that the recommended procedure for reduction and carboxymethylation releases physicochemically very similar if not identical J chain populations from all tested biological fluids Furthermore the procedure seems to release the entire J chain population from its companion polypeptide chains in all tested fluids since no further release was observed either when higher concentrations of the reducing substance were used or when the reduction and carboxymethylation were allowed to take place in a strongly denaturing buffer Additional support for a complete J chain release is given by the observation that the hydrolysate of the J chain population released by the recommended procedure from a 19S IgM M component contains only carboxymethylcysteine and no trace of cystine indicating that the J chain population has had all its half cystine residues transformed to carboxymethylcysteine Also in agreement with this result is the observation that the electrophoretic mobility of the J chain population released from a 19S IgM M-component by the recommended procedure is identical with that of the J chain population released from the M-component on reduction and carboxymethylation in a strongly denaturing buffer

Since the J chain populations released from all tested biological fluids seemed to be physicochemically identical it would have been simple to construct an immunochemical method for J chain quantitation with good accuracy provided the J chain concentrations were expressed in relative values However since it was desirable to express the J chain concentration in absolute molar amounts a reasonably pure J chain standard was required which firstly had physicochemical properties identical with those of the J chain populations released from the biological fluids and secondly was pure enough to allow a correct physicochemical quantitation These two requirements were found to conflict since the procedures necessary for the production of a completely pure J chain preparation invariably resulted in a more or less polymerized J chain population However by repeated gel filtrations and by avoiding freezing and thawing lyophilization and temperatures above 25°C it was found possible to produce a standard with a J chain population physicochemically identical with the J chain populations released from all tested biological fluids containing not more than 5–10% contaminating proteins and thus allowing a reasonably accurate physicochemical quantitation

Since errors of up to several hundred per cent were involved in the immunochemical quantitation

of monomeric J chain populations in test samples with the use of a 99% pure but partly polymerized J chain population in the standard it was obviously preferable to accept the small error in the physico chemical determination of a 90-95% pure mono meric J chain standard

Although the monomeric J chain standard solution could be kept at −24 C for at least 6 months and then thawed at room temperature without polymerization the limited amount of this J chain preparation made it convenient to use a secondary J chain standard solution for the immunochemical J chain quantitation A β lipoprotein-depleted (6) human plasma containing a highly concentrated 19S IgM M-component was used for this purpose This plasma could repeatedly be frozen and thawed and stored for long periods at +6°C with no decrease in the release of a monomeric J chain population on *reduction and alkylation*

An electroimmunoassay system was chosen to compare the reaction of the antiserum with the standard and with the test samples since this method is sensitive and rapid and most important the features of the precipitates often change notably with small alterations in the antigenic molecular populations such as those caused by limited polymerization or degradation of the antigen molecules (20)

The dilution step of the procedure for J chain quantitation which decreases the sensitivity was introduced since it was observed that reduction of undiluted human plasma resulted in coagulation This phenomenon was probably caused by the high albumin concentration in plasma and did not occur when biological fluids such as saliva having low albumin concentrations were reduced

Reduced and alkylated test and standard samples were used for the immunoglobulin determinations in human plasma saliva and milk samples to obtain similar molecular weight distributions in all samples Single radial immunodiffusion and antisera raised against polyclonal immunoglobulin populations were used for the immunochemical determinations proper since this combination was thought to be minimally influenced by the various differences in antigenic and charge properties of the M-components in some of the test samples This supposition was tested for two pure 19S IgM M-components by dissolving a specified amount of them in buffer and then determining their concentration by the *immunochemical system described* above The results agreed within 10% with those expected

For both normal human milk and saliva the J chain/7S IgA molar ratio was found to be close to 0 5 with a comparatively small variation between different saliva and milk samples The figures are probably only slightly influenced by J chain released from IgM since the IgM concentrations in the milk and saliva samples were very low Assuming that the predominant IgA species in fresh normal human milk and saliva is the dimeric secretory IgA the figure 0 5 agrees with the results of Halpern and Koshland (12) and Mestecky et al (25) who purified secretory IgA and demonstrated by physicochemical procedures the presence of one J chain in each IgA dimer

When the J chain/7S IgA molar ratios in 92 plasma samples with highly concentrated IgA M components were calculated 49 samples were found to have ratios close to zero while the remaining samples had varying ratios up to about 0 5 The highest ratio obtained was 0 64 Preliminary investigations by gel chromatography on columns of Sephadex G 200 of two plasma samples with J chain/7S IgA ratios close to zero and of two samples with ratios around 0 5 showed that the IgA M-components devoid of J chains were monomeric while the IgA M components with J chain/7S IgA ratios of about 0 5 consisted predominantly of dimers These results are concordant with those of Halpern and Koshland (12) and Mestecky et al (25) who investigated the J chain content of purified monomeric and dimeric IgA by physicochemical methods The gel chromatographic studies also indicated that while the monomeric J chain free IgA formed complexes with other plasma proteins such as albumin α_1-antitrypsin and protein HC (32) no such complexes were formed with the dimeric J chain rich IgA This inverse relation between the amount of plasma protein IgA complexes and the J chain/IgA molar ratio agrees with earlier results (21)

When plasma samples containing concentrated IgG of IgD M-components were tested for their J chain concentration by the method described in this work no evidence was found for the association of J chain with these Ig classes This was also the case when a concentrated solution of a pure 8S IgM M-component was investigated These results agree with those obtained by physicochemical [illegible] of *purified immunoglobul*[illegible] 25)

When the J chain/19S IgM molar ratios in 33 plasma samples with highly concentrated IgM M-components and low amounts of IgA were calculated a mean value of 1 8 was obtained The range of all values was 1 0-2.4 *The mean ratio of 1 8* differs from the values obtained by Chapuis and Koshland (9) and by Mestecky et al (25) who *purified 19S IgM M-components and determined* their J chain/IgM molar ratio to be about 1 0 by physicochemical means Although polymeric IgA in *the plasma samples may release some J chain and* thus increase the values obtained by the present immunochemical procedure this cannot explain the *entire discrepancy The difference may obviously* be explained by systematic errors in the immunochemical or physicochemical methods but an ex*planation compatible with all above mentioned data* may also be offered since IgM molecules may contain two different J chain populations one of which *is covalently attached in a 1 1 ratio while the other* is non-covalently bound and therefore may be removed during purification procedures Komar and Mukkur (16) *have demonstrated that bovine co*lostral IgM is associated with two J chain populations of approximately equal sizes and that only one of these is covalently bound (in a *1 1* molar ratio) to the IgM molecules

No plasma samples with concentrated IgM M-components *were found to have such low J* chain concentrations that the presence of an -component devoid of J chain could be suspected *three plasma samples with IgM M-components* precipitation patterns in the electroim- which differed markedly from those *usually obtained being diffuse and badly de*marcated These precipitates were not used for quantitation since altered precipitation patterns de*note altered J chain populations and therefore* signify physicochemical differences between the J chain populations in the standard and test sam*ples which may greatly decrease the accuracy of* immunochemical quantitations The occurrence of the anomalous precipitation patterns may have *been caused by proteolytic activity in the three* samples since such activity may bring about degradation of the J chain in polymeric IgM (18)

ACKNOWLEDGEMENTS

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Lymphoma of the Small Intestine in Adult Coeliac Disease

L Brandt B Hagander Å Norden and M Stenstam

From the Department of Internal Medicine University Hospital Lund Sweden

ABSTRACT Five out of 74 patients with adult coeliac disease (ACD) diagnosed from 1965 through 1977 developed intestinal lymphoma The age at diagnosis of ACD was 54–63 years (mean 58) and in all patients an initial improvement was noted on a gluten free diet The interval between the diagnoses of ACD and lymphoma was 0 5–6 years (mean 3) In 4 patients the diagnosis of lymphoma was preceded by relapse of diarrhoea and loss of weight despite an adequate diet 2 patients developed perforation of the bowel In all patients who subsequently developed lymphoma a pronounced lymphocytopenia 0 4–1 3×10⁹/l, was found at the diagnosis of ACD It is suggested that ACD patients over 40 who have relapse of malabsorption symptoms despite an adequate diet should be investigated for the presence of an intestinal lymphoma The suspicion of a malignant transformation may be especially strong if a previous pronounced lymphocytopenia has been recorded Three patients died 1–12 months after the diagnosis of lymphoma However 2 patients are in good condition 14 and 20 months respectively, after laparotomy and removal of the tumour, indicating that an early diagnosis and treatment may have a reasonably palliative effect in some cases

A high incidence of malignant lymphoma originating in the small intestine in adult coeliac disease (ACD) has been reported in several studies (1 2 7 11) In an attempt to find out whether some clinical signs might herald the development of intestinal lymphoma in ACD the clinical course has been analysed in 5 patients with ACD diagnosed prior to the intestinal malignancy

It has previously been demonstrated that lymphocytopenia is common in ACD (4) and that a deficiency in T lymphocytes is characteristic at diagnosis (9) Since a deficiency in T-cells may have some bearing on the development of lymphoma (5) special interest has been devoted to the lymphocyte counts prior to the diagnosis of lymphoma

PATIENTS AND METHODS

The case records of all 74 patients with ACD diagnosed between the ages of 14 and 73 (mean 42) at our department from 1965 through 1977 were examined In all patients biopsy specimens from the jejunal mucosa obtained with a Crosby capsule showed a flat mucosa characteristic of coeliac disease Lymphocyte counts were calculated from WBC counts and differential counts

RESULTS

In 5 of the 74 patients intestinal lymphoma was diagnosed at various intervals after the diagnosis of ACD Clinical data on these patients are given in Table I The age of these 5 patients at diagnosis of ACD was 54–63 years (mean 58) which is considerably higher than the mean age of 42 years for all of our ACD patients

An initial improvement in the symptoms was noted in 4 patients (nos 1 2 3 and 5) on a gluten free diet followed later by recurrence of symptoms resistant to dietary treatment One patient (no 4) did not keep to a diet In 2 patients (nos 4 5) there was a sudden onset of abdominal symptoms due to perforation of the bowel

The time from diagnosis of ACD to diagnosis of intestinal lymphoma was 0 5–6 years (mean 3) and the age of the patients at diagnosis of the malignant tumour was 56–65 years (mean 61)

The lymphomas were reported by the pathologist as reticulosarcoma or more recently due to a change in nomenclature histiocytic lymphoma

In a previous study (4) the median lymphocyte count in a healthy control group was 2 1×10⁹/l In a group of 30 ACD patients followed for 1–12 years (mean 5 5) without developing signs of any malignant tumour the median lymphocyte count at diagnosis was 1 4×10⁹/l In the patients who subsequently developed lymphoma the lymphocyte counts at diagnosis of ACD were low the range being 0 4–1 3×10⁹/l (Fig 1)

Table I *Clinical data on 5 ACD patients developing intestinal lymphoma*

Case no	Sex	Year of birth	ACD symptoms	Age at and year of diagnosis of ACD	Response to dietary treatment	Symptoms of intestinal lymphoma
1	♂	1916	Lassitude borborygmus oedema started 1961	55 1971	Improved for 3 mo	Oct 1971 return of diarrhoea abdominal pain increasing oedema ascites skin tumours
2	♀	1914	Diarrhoea started 1967	54 1968	Good	March 1974 return of diarrhoea oedema weight loss fever March 1975 peritonitis
3	♀	1905	*Abdominal pain lassitude 1948* Borborygmus diarrhoea oedema 1967	63 *1968*	*Improved for 1 y*	May 1969 return of diarrhoea abdominal pain weight loss Aug 1969 fistula between small intestine and bladder Sept 1969 laparotomy
4	♂	1912	Dermatitis herpetiformis diagnosed 1971 Jejunal biopsy 1971 flat mucosa	59 1971	Did not keep strict diet	March 1976 peritonitis Laparotomy
5	♂	1913	Diarrhoea started 1973	61 1974	Good	Sept 1976 return of diarrhoea abdominal pain weight loss Dec 1976 peritonitis Laparotomy

DISCUSSION

The finding of 5 cases of intestinal lymphoma ong 74 ACD patients is comparable to the inci nce of 6–10% previously demonstrated (1 2 7 1) and confirms that malignant lymphoma is unduly common in ACD All lymphomas were classified as reticulosarcoma (histiocytic lymphoma) which was also a common diagnosis in patients previously reported (8 11)

*Although it has been suggested that malabsorp*tion symptoms similar to ACD and the characteristic flat mucosa may be secondary to an intestinal lymphoma (3 10) there are strong indications that intestinal lymphoma should be regarded as a complication of ACD (1 6 7) In the patients with flat mucosa and intestinal lymphoma described by Harris *et al* (7) the duration of malabsorption symptoms averaged 21 years suggesting that ACD preceded the development of malignant lymphoma Our patients responded satisfactorily to a gluten free diet indicating that their symptoms of malabsorption were caused by coeliac disease

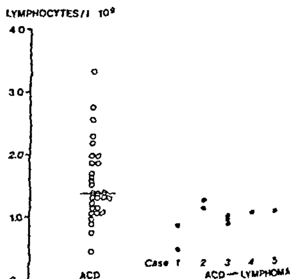


Fig 1 Lymphocyte counts at diagnosis of ACD in 30 patients who have not developed lymphoma and in 5 patients who subsequently developed intestinal lymphoma. The lymphocyte counts in the lymphoma group were determined on 1–3 occasions in each patient

ype of mphoma	Treatment	Outcome
ticulosarcoma necropsy)	Short course of cyclophos phamide	Died 1 mo after return of diarrhoea
ticulosarcoma necropsy)	–	Died of peritonitis one y after return of diarrhoea
ticulosarcoma in ransverse colon nd small intestine vith fistula to the ladder	Surgery ^{60}Co irradiation	Died one y after return of diarrhoea
istiocytic lym homa 10 cm below g Treitz	Surgery	Alive 20 mo after operation
istiocytic ymphoma	Surgery	Alive 14 mo after operation

rather than by a malignant lymphoma This interpretation is in line with the findings in patients 1 2 3 and 5 who shortly before the diagnosis of malignant lymphoma had a relapse of diarrhoea despite an adequate dietary regimen It is probable therefore that in our patients malabsorption symptoms due to ACD responded to the dietary treatment whereas the similar symptoms caused by tumour growth did not Our clinical data accordingly support the concept that the intestinal lymphoma is secondary to ACD The period of 3 years which elapsed between the first appearance of malabsorption symptoms and symptoms of a malignant intestinal tumour in case 5 suggests that the development of lymphoma may start shortly after the manifestation of ACD and that there need not be a lengthy period of coeliac disease for the development of intestinal lymphoma

Lymphocytopenia is a characteristic trait at diagnosis of ACD (4) and low numbers of T lymphocytes is a common finding (9) In the patients who developed intestinal lymphoma extraordinarily low lymphocyte counts were recorded at the diagnosis of ACD There is experimental and clinical evidence that a deficiency in T lymphocytes may favour the development of lymphoma (5) and it is possible therefore that the pronounced lymphocytopenia noted in our patients may have some bearing on their subsequent lymphoma

Among the patients described by Austad et al (1) 16 out of 17 with known villous atrophy were more than 40 years old and among those reported by Harris et al (7) 6 out of 8 were 40 years or older at the diagnosis of lymphoma In the present patients the lymphomas were diagnosed at even higher ages i e at 56–65 years It therefore seems that the risk of intestinal lymphoma is most pronounced in the elderly ACD patients and that such tumours are less likely to develop in young patients with ACD Recurrence of diarrhoea and loss of weight despite a gluten free diet are common symptoms heralding the diagnosis of intestinal lymphoma (1 7) and they were also characteristic symptoms in 4 of our patients Another typical finding in our patients was as mentioned the pronounced lymphocytopenia at diagnosis of ACD We therefore suggest that ACD patients over 40 who have a relapse of malabsorption symptoms in spite of an adequate dietary regimen should be carefully investigated for the presence of an intestinal lymphoma The suspicion of malignant transformation may be especially strong if a previous pronounced lymphocytopenia has been recorded Patients 4 and 5 in whom laparotomy and excision of the tumour were performed due to bowel perforation are in good condition 20 and 14 months respectively after the operation In some patients removal of the tumour may therefore give a reasonably palliative effect and diagnostic efforts including laparotomy to obtain an early diagnosis of intestinal lymphoma in ACD patients may not be futile

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Interferon Therapy in Neoplastic Disease

A Preliminary Report

Ivan Strøyer Christophersen Robert Jordal Kurt Osther
John Lindenberg Poul Hjortkjær Pedersen and Kurt Berg

From the Department of Oncology and Radiotherapy and the Department of Gynecology and Obstetrics Herlev University Hospital Herlev the Department of Clinical Chemistry Gentofte University Hospital Gentofte the Department of Surgery Frederiksberg Hospital Copenhagen and the Institute of Microbiology Århus University Århus Denmark

ABSTRACT The treatment of 10 patients having neoplastic disease with exogenous i m interferon therapy is described The interferon given is partially purified interferon produced from human leukocytes Sendai virus is used as interferon inductor The patients reported in this paper have been on treatment for periods of 2–28 months Apart from initial periods of fever, no side-effects have been recorded Patients suffering from bladder papillomas have shown partial regression after a few months of therapy The other cases treated are too few to warrant any conclusions but the therapy does seem to have been beneficial

Key words Interferon neoplasia treatment papilloma of bladder

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In 1957 Isaacs and Lindenmann (8) found interferon to be a substance with antiviral effect They showed that cells infected by virus produced a protein which protects uninfected cells against virus attack This protein was called interferon

Recent studies have demonstrated that interferon possesses immune regulatory properties (5) Other works have demonstrated interferon induced inhibition of cell growth in in vitro investigations (16) The cell growth inhibiting property of interferon has also been demonstrated in vivo by treating mice suffering from leukemia or malignant lymphomas with resulting remission (4 6) Several works from Sweden and Finland have shown that high doses of interferon administered for long periods to patients with malignant diseases are effective and no serious side-effects have been recorded (2 11 12 13 14 15 17)

Due to the limited availability of interferon only neoplastic diseases with extremely bad prognosis such as osteosarcoma (12) one case of Hodgkin s disease (2) a few cases of myelomas and papillomas in larynx (juvenile type) (personal communication) have been reported Until now interferon therapy given for long periods has not shown serious side effects (1 3 14 15)

Although we have not treated a great many cases to date we have found it imperative to report our experiences from interferon therapy

MATERIAL AND METHODS

Interferon

The interferon was prepared from human leukocytes as described by Mogensen and Cantell (9) and subjected to partial purification by the selective precipitation of contaminating proteins from a 94% ethanolic solution (9) The preparations used for therapy contained about 4 mill interferon units (IFU) per ml and had a protein content of approximately 0 5 mg/l mill IFU corresponding to $1–2\times10^6$ IFU/mg protein

Interferon treatment

Interferon was administered as a partially purified interferon (P IF) i m as described by Strander et al (13 14) The doses employed are stated in Results

Testing of the interferon preparations

The interferon preparations were tested for activity on U-cells using plaque technique (14) The protein bands were checked on SDS PAGE and compared to P IF preparations provided by Dr K Cantell Cell inhibition effect was tested on Daudi cells (7) The preparations were checked for sterility and pyrogenicity

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Abbreviations IFU=interferon unit P IF=purified interferon

CASE REPORTS

Patient 1

A 16-year-old girl with osteosarcoma positioned in the distal part of left femur She was treated initially 1 2 years ago with amputation of left femur The patient had no clinical signs of metastases Interferon treatment was commenced with P IF 4 mill IFU i m daily for one month starting immediately prior to operation followed by P IF injections 3 times a week to be continued for a total of 1.5 years

Cytostatic regimen

Four weeks after surgery the patient was treated for 6 weeks with high-dose methotrexate 3 000 mg per course followed by citrovorum rescue factor

Patient 2

A 29 year-old man with an osteosarcoma in the proximal part of humerus He was subjected to an amputation of the right humeroscapular joint 3 6 years ago Left lung metastases were detected 6 months after the operation and thoracotomy with exirpation of metastatic tissue was performed Pulmonal metastases were found after this operation P IF treatment with 2 mill IFU i m was started 3 years ago three times a week followed 1 year ago by 4 mill IFU daily for one month since then 3 doses a week Treatment is continuing

Cytostatic regimen

Combined cytostatic courses consisting of high-dose methotrexate Adriamycin and vincristine were started 3 1 years ago

Patient 3

An 8-year-old boy with an Ewing sarcoma localized to the right tibia (proximal part) Initial treatment was high voltage irradiation (2 36 Gy/20F 4F/w 82 TDF CRE 1555 reu) The patient was given interferon 4 mill IFU immediately after irradiation daily doses for 14 days and thereafter 3 weekly doses Until now the patient has been on treatment with interferon for 1 year and is to continue treatment for 1 5 years

Cytostatic regimen

Combined cytostatic treatment was started at the time of irradiation The treatment consisted of dactinomycin Oncovin cyclophosphamide and Adriamycin 4 courses were given at intervals of 2 months

Patient 4

A 61 year-old woman suffering from a synovial sarcoma She was treated with amputation of distal part of left femur Immediately after surgery the patient was given interferon 4 mill IFU daily for one month followed by 4 mill IFU 3 times a week She has been on treatment for half a year and is to continue for 1 5 years

Cytostatic regimen

None

Patient 5

A 34 year-old woman suffering from an osteosarcoma positioned in the middle of the left humerus She was subjected to amputation of left humeroscapular joint 1? months ago No metastases were recorded Starting 3 months ago the patient was given interferon 4 mill IFU daily for one month and 3 times a week during the following two months

Cytostatic regimen

Adriamycin 60 mg/m every three weeks until a total dose of 450 mg/m² had been given before admission to our department

Patient 6

A 47 year-old woman suffering from malignant mesenchymoma in the middle part of left femur Three months ago the patient was subjected to local resection She received postoperative high voltage irradiation (3 0 Gy/15 F 4 F/w 76 TDF CRE 1590 reu) Starting 2 months ago interferon is being administered 4 mill IFU daily for one month and thereafter 3 times weekly

Cytostatic regimen

Fourteen days after surgery the patient was given Adriamycin 60 mg/m² every 4 weeks Scheduled total dose is 450 mg/m² Cyclophosphamide 150 mg daily for 7 days every 4 weeks

Patient 7

A 66-year-old woman had been subjected to explorative pyelotomy 6 years ago because of papillomas in renal pelvis (grade II) Nephroureterectomy (left side) was performed 3 years ago because of complete papillomatous degeneration in renal pelvis

Until 1 year ago the patient was treated several times with electrocoagulation because of recidivating multiple papillomas in the bladder One year ago interferon treatment was started with 4 mill IFU daily for 2 months followed by 4 mill IFU 3 times a week The treatment is to be continued for a total of 1 5 years

Cytostatic regimen

None

Patient 8

A 54-year-old man in whom treatment with electrocoagulation was initiated 4 years ago because of papillomas in the bladder (grade I) Until half a year ago the patient was electrocoagulated several times for multiple papillomas Interferon treatment was started half a year ago at the same doses as given to patient 7

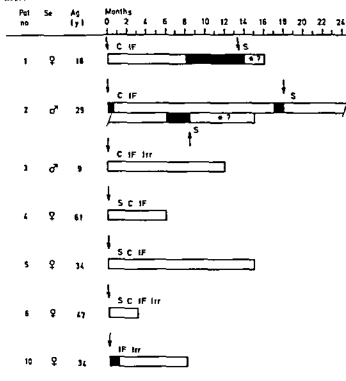
Cytostatic regimen

None

Patient 9

A 46-year-old woman who has been suffering for 7 years from widespread papillomas (grade II) in the bladder and been electrocoagulated several times Interferon treatment was started with 4 mill IFU daily for one month and

Table I *Results of interferon therapy in patients 1–6 with bone sarcomas and patient 10 with cancer colli uteri*



□=Periods without metastases ■=periods with metastases *?=no clinical signs of metastases S=surgery C=cytostatic treatment started IF=interferon treatment started Irr =high voltage irradiation

3 times a week during the following months The patient has been on interferon treatment for 7 months

Cytostatic regimen
None

Patient 10

A 34-year-old woman with a cancer colli uteri stadium II B The patient was given high voltage irradiation on gynecological areas (39 50 Gy/10 F 2 F/w TDF 82 CRE 1560 reu) Local cobolt irradiation using afterloading principle At the time of irradiation interferon treatment was started with 4 mill IFU daily for one month followed by 3 times a week and the patient has now been on interferon treatment for 8 months

Cytostatic regimen
None

RESULTS

The number of patients treated is too small and the observation periods during treatment are too short for definite conclusions about the effect of interferon on cancer But the following results can be stated Patients 3–6 have not developed any metastases to date (Table I)

Patient 1 developed lung metastases 8 months after the start of interferon treatment Thoracotomy was performed but was not radical After a short pause she has continued with interferon treatment Furthermore high-dose methotrexate is scheduled

Treatment of patient 2 with interferon and cytostatics was started during his first metastatic period (Table I) and he was brought into remission for a period of 17 months Relapse reappeared in the lungs and (radical) thoracotomy was performed Still under interferon treatment the patient obtained remission for a further 12 months He then developed a solitary metastasis in thoracal vertebra VIII and paresis of both legs Laminectomy for decompression was performed After a short pause the interferon treatment was continued The paresis disappeared shortly after operation and the patient has now had a period of 7 months without any progression in his disease

After treatment for a few months with interferon

patients 7 8 and 9 showed normalization of their bladder urothelium Regression of papillomas smaller than 1 cm was detected whereas those larger than 1 cm were stationary Patient 10 (Table I) who had a stage 2B collum cancer before irradiation and interferon treatment has been without local recurrence or detectable metastases for 8 months

DISCUSSION

In recent years interest in the clinical use of interferon has grown considerably Relatively few cases have been published which reflects the limited availability of interferon Our experience with interferon based on the 10 patients described is that it may be utilized for long term treatment without any other serious side-effects than fever during the initiation of treatment No allergic manifestations have been observed We have no explanation for the initial fever though it may be directly associated with the working mechanism of interferon (10)

Hospitalization is needed only at the initiation of treatment with interferon which may otherwise be administered at home The size of the dose is approximately that used by Strander et al 4×10⁶ IFU (personal communication) Only further clinical investigations can reveal any benefit of higher doses The reasons why we have given interferon therapy to patients with bone sarcomas are that trander et al have found this therapy to be effec on osteosarcoma (14) and that the growth of eosarcoma cells in tissue culture seems to be inhibited by interferon doses corresponding to the concentrations in the patients (16) Strander et al found that 64% of patients treated with interferon show no metastases after 2½ years compared with 32% of the control group and that 73% of patients were still alive after 2½ years of interferon treatment compared with 35% of the control group (14) Based on these facts we consider that interferon treatment should always be used in the primary treatment of patients with osteosarcomas and that the treatment should be initiated shortly after establishing the diagnosis In our patient 2 with osteosarcoma and lung metastases we started treatment with interferon under this condition

During combined treatment with cytostatics and interferon we succeeded in bringing the patient into remission for a period of 17 months This long period of remission is noteworthy compared to the general fate of patients suffering from osteosarcomas with lung metastases (14) The next metastasis situated in VIII vertebra as a sole tumour appeared after an interval of 12 months It was treated as described and interferon treatment was started again with daily doses of 4 mill IFU Furthermore it is of interest that 7 months after the last surgery the patient shows no sign of progression in his disease Thus it may be possible to begin interferon treatment with some benefit for the patient even though lung metastases have developed

The patient group referred to (14) has only received interferon therapy For 5 out of 6 patients with bone sarcomas we have chosen to combine the interferon treatment with cytostatics The reason why we have given interferon to three patients with bladder papillomas and to one patient with cancer colli uteri is that virus might be a causal agent for these types of neoplasms In all three patients regression was found in the small papillomas but not in the large Interestingly enough we have not seen any exacerbation of the papillomas Interferon may be useful for example in the therapy of patients suffering from bladder papillomas

One patient suffering from cancer colli uteri received interferon as an adjuvant therapy

Further randomized investigations with interferon in this disease are required The effect of interferon treatment on cancer will be investigated in larger scale systematic studies

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Interferon and Spontaneous Cytotoxicity in Man

II Studies in Patients Receiving Exogenous Leukocyte Interferon

Stefan Einhorn Henric Blomgren and Hans Strander

From Radiumhemmet Karolinska Hospital Stockholm Sweden

ABSTRACT The spontaneous cytotoxicity of peripheral lymphoid cells from five tumor patients was measured before and at various times after the first injection of human leukocyte interferon (IF) Four of the patients lymphocytes exhibited cytotoxicity before the IF injection After injection of IF there was an initial decrease in cytotoxicity, followed by an increase to 1 5–5 times above the preinjection level the peak being reached at 12 hours Thereafter the spontaneous cytotoxicity decreased but usually remained elevated for 24 hours after the injection The lymphocytes of the fifth patient had very low spontaneous cytotoxicity before the injection of IF and this did not markedly change afterwards The proportion of E-rosette forming cells seemed to decrease slightly in all patients after the injection followed by a normalization at 24–48 hours

Key words Interferon lymphocytes killer cells
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Since the original characterization of interferon (IF) in 1957 (11) IF preparations have been shown to exert a number of effects in addition to antiviral effects such as inhibition of cell multiplication (17) and interaction with various immunological functions (16) In animal systems IF is a potent antitumor agent for chemically and virally induced tumors as well as for transplanted and spontaneously occurring tumors (8)

Although IF preparations have been shown to exert antitumor effects at least in animal systems it is still not established in what ways they act One possibility is a direct effect on the tumor cells another is that IF enhances an antitumor activity of the host The natural killer cell system has been suggested to be of importance in the host defense against tumors in mice (9 15) and it has recently been shown that the spontaneous cytotoxicity of human lymphocytes is enhanced when the lymphocytes are treated with IF in vitro (6 25)

Since 1969 more than 100 patients suffering from various neoplastic diseases have been given human leukocyte IF at the Karolinska Hospital (22) A trial of the effect of IF in patients with osteosarcoma was initiated in 1971 (1) and trials in cases of laryngeal papilloma and myeloma were initiated in 1976 In the present work we have studied the effect of a single injection of human leukocyte IF on the spontaneous cytotoxicity of peripheral lymphocytes from five tumor patients We have also studied whether the cellular composition of the peripheral lymphocyte population is altered by IF administration

PATIENTS

Case 1 is a 23 year-old male with a laryngeal papilloma a benign tumor of the larynx He has had the disease since the age of 2 and has been operated on more than 20 times prior to the onset of IF therapy The patient developed a fever of 38 C after the injection of IF

Case 2 is a 66-year-old female with a multiple myeloma with no treatment for the disease prior to IF injection She developed a fever of 40°C a few hours after the injection of IF

Case 3 is a 20-year old male with a grade IV osteoblastic osteosarcoma of the right humerus with no treatment for the disease prior to IF injection He had pain at the site of injection and developed a fever of 38 C after the injection of IF

Case 4 is a 21 year old male with a grade IV osteoblastic osteosarcoma of the left femur He had received no treatment for the disease prior to the IF injection No fever or local pain developed after the injection of IF

Case 5 is a 14 year-old male with a grade III osteoblastic

Abbreviations IF = interferon MEM = Eagle s Minimal Essential Medium supplemented with Earle s SRBC = sheep red blood cells

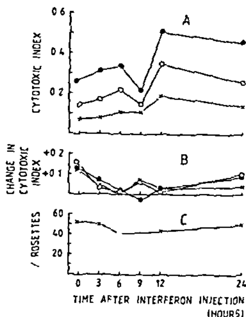


Fig 1 Case 1 *(A)* Spontaneous cytotoxicity before and at various times after IF injection Lymphocyte target cell ratios 25 1 (×---×) 50 1 (O---O and 100 1 (●---●) were used *(B)* Change in cytotoxicity after addition of IF in vitro as tested before and at various times after IF injection Lymphocyte target cell ratios see above *(C)* Per cent E rosettes before and at various times after the injection of IF

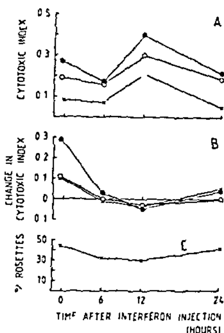


Fig 2 Case 2 Text and symbols as in Fig 1

osteosarcoma of the left femur with no treatment for the disease prior to the IF injection He developed a fever of 39°C after the injection of IF

L Å Brostrom S Haglund and H Mellstedt provided patients for this study In one experiment lymphocytes from a 22 year-old healthy male were used as controls

METHODS

Interferon preparations

The IF preparations supplied by K Cantell were derived from human peripheral blood leukocytes exposed to Sendai virus as previously described (3) The osteosarcoma patients received concentrated IF preparations with a specific activity of approximately 2×10⁴ IF units/mg of protein The remaining two patients received partially purified IF with a specific activity of approximately 1×10⁶ IF units/mg of protein The antiviral activities of the preparations were determined by assaying inhibition of plaques induced by vesicular stomatitis virus in U-cells (23) The antiviral activity is expressed in international units by comparison with the international reference preparation 69/19

Lymphocyte preparations

Lymphoid cells were separated from heparinized venous blood by centrifugation of Ficoll Isopaque (13) The cells were then washed twice by centrifugation in Eagle s Minimal Essential Medium supplemented with Earle s salts (MEM) Approximately 80–90% of the cells were classified as lymphocytes when assessed after crystal violet staining the rest being classified as monocytes or granulocytes This lymphocyte preparation was used for the cytotoxicity tests

In one patient (case 4) the lymphoid cells were separated into two fractions using a rosette sedimentation technique (12) Briefly the lymphoid cell suspensions were first treated with iron powder and a magnet was used to remove phagocytic cells The cells were then incubated with sheep red blood cells (SRBC) and thereafter centrifuged on Ficoll Isopaque The sedimentating cells i e those forming spontaneous rosettes with SRBC will be termed T-cell enriched The cell fraction at the fluid interface will be termed non T cell enriched

Rosette formation

T-cells were identified by their capacity to bind SRBC as described before (2) Cells forming rosettes with SRBC will be termed E rosette forming cells Lymphocytes possessing membrane receptors for the Fc part of IgG or activated C3 were identified according to the method described by Jondal (12) with the modification that the indicator cells used for detection of Fc receptor positive cells were ox RBC treated with rabbit 7S anti-ox RBC and indicator cells used for detecting C3 receptor bearing cells were treated with 19S anti-ox RBC followed by incubation in mouse serum as a source of complement (strain A CA) Cells binding these indicator cells will be termed EA and EAC rosette forming respectively Spontaneous killer cells in the blood have been identified as Fc receptor bearing non T lymphocytes (5 7 14 18)

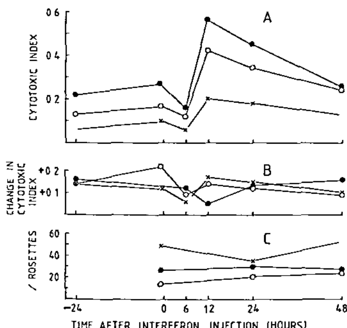


Fig 3 Case 3 (*A*) (*B*) Text and symbols as in Fig 1 (*C*) Per cent E rosette forming cells (× ×) EA rosette forming cells (O O) and EAC rosette forming cells (● ●) before and at various times after the injection of IF

Cytotoxic assay

Chang cells from human liver were used as target cells in all experiments but one in which the osteosarcoma cell line 393T (19) was used in addition to Chang cells (patient 4)

One million target cells suspended in 0 5 ml of MEM containing 10% of heat inactivated human serum were labelled with 0 1 ml of $Na_2^{51}CrO_4$ (75 μCi specific activity 100–350 μCi/μg Cr the Radiochemical Center Amersham England) for 1 h at 37 C They were then washed four times by centrifugation and resuspended in MEM with 10% of human serum Ten thousand labelled cells were then transferred to Ependorf reaction tubes (NO 3810 Ependorf Geratenbau Netheler + Hinz GmbH Hamburg W Germany) together with various numbers of lymphocytes and the volume was adjusted with medium to 0 6 ml To some of these tubes IF was added at a final concentration of 30 units/ml Spontaneous release was determined in tubes which received only target cells and medium After centrifugation at 200 *g* for 1 min the cells were incubated for 4 hours at 37 C The radioactivity of 0 2 ml of the supernatant and of the remaining 0 4 ml was determined using a gamma counter and expressed as cpm

Percent release was determined according to the formula

$$\frac{3 \times \text{cpm of 0 2 ml supernatant} \times 100}{\text{cpm of 0 2 ml supernatant} + \text{cpm of remaining 0 4 ml}}$$

A cytotoxic index was calculated according to the formula

$$\frac{\text{\% release with lymphocytes} - \text{\% spontaneous release}}{100 - \text{\% spontaneous release}}$$

Arithmetic mean values of duplicates were calculated The mean variability within the duplicates was 2% The numerical difference in cytotoxic index between tubes containing lymphocytes target cells and 30 units of IF/ml and tubes containing lymphocytes and target cells only expresses the change in cytotoxic index by IF as 30 units of IF/ml alone had no effect on the Cr^{51} release of the target cells

Experimental design

Three million units of IF were injected intramuscularly in each patient at 9 a m The spontaneous cytotoxicity and rosette forming ability of the patients lymphocytes were tested before and at various times after the first IF injection Immediately after the blood had been drawn the lymphocytes were separated and incubated with ^{51}Cr labelled target cells Preliminary experiments showed that the capacity of lymphocytes to form rosettes was not altered to any detectable extent by storage at 4 C for 48 hours The lymphocytes obtained at different times were therefore stored at 4 C and tested for formation of rosettes simultaneously after 24–48 hours

RESULTS

Spontaneous cytotoxicity

Before IF injection the lymphocytes of patients 1 2 3 and 5 exhibited spontaneous cytotoxicity exceeding 20% with the highest lymphocyte target cell ratio tested 100 1 (Figs 1*A* 2*A* 3*A* and 5*A*) A slight decrease in cytotoxicity was observed in these patients 3–9 hours after the injection thereafter the cytotoxicity increased in all cases Of the intervals tested the highest level was observed at

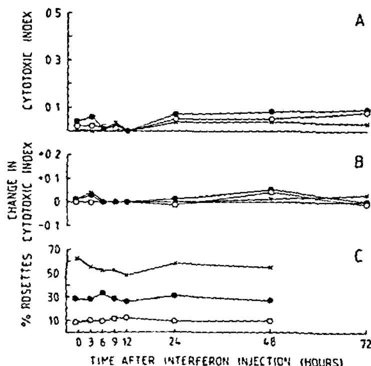


Fig 4 Case 4 Text and symbols as in Fig 3 A second injection of IF was given 48 hours after the first

12 hours and was then 1 5–5 times above the preinjection level Cytotoxicity then decreased but was still elevated 24 hours after the injection in patients 1 3 and 5 The lymphocytes of patient 4 exhibited very low cytotoxicity before IF injection and this activity was not changed thereafter to any major extent (Fig 4*A*) Not even a second IF injection given at 48 hours augmented the cytotoxicity of his lymphocytes (Fig 4*A*) In this patient cytotoxicity was not observed when cells also from the osteosarcoma cell line 393T were used as target at 48 hours after the first injection of IF (data not presented)

Before injection of IF the spontaneous cytotoxicity of the lymphocytes of patients 1 2 3 and 5 could be augmented by IF in vitro (Figs 1*B* 2*B* 3*B* and 5*B*) It was of interest to examine whether the enhanced cytotoxicity of the patients lymphocytes after IF injection could be further increased by treatment with IF in vitro Twelve hours after the injection of IF, the capacity of IF to augment cytotoxicity in vitro seemed to be reduced in two patients (Figs 1*B* and 2*B*) but remained at the same level in two others (Figs 3*B* and 5*B*) In case 4 there did not seem to be any augmentation of the cytotoxicity by IF in vitro at any of the times tested

Table 1 Proportion of E EA and EAC rosette forming cells (%) in unfractionated T and non T-cell enriched lymphocyte preparations in patient 4 and an age and sex matched control

The lymphocytes were separated from the patient s blood 48 hours after the first IF injection (Fig 4)

	Lymphocyte preparation	E rosette forming cells	EA rosette forming cells	EAC rosette forming cells
Control	Unfractionated	54	28	24
Patient 4		55	10	27
Control	T-cell enriched	88	9	5
Patient 4		87	5	12
Control	Non T-cell enriched	15	51	49
Patient 4		35	11	48

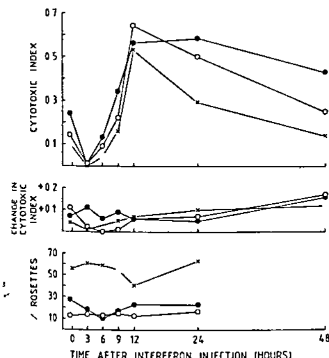


Fig 5 Case 5 Text and symbols as in Fig 3

Lymphocyte subpopulations

Administration of IF in vitro did not result in any major change in the proportions of EA and EAC rosette forming lymphocytes (Figs 3C 4C 5C) Small reductions in the proportions of E rosette forming cells were observed followed by a normalization at 24–48 hours (Figs 1–5(C)) but the number of patients is too small to allow any conclusions

Since spontaneous killer cells in the blood are considered to be Fc receptor bearing non T lymphocytes it was of interest to examine whether the EA rosette forming cells of patient 4 who displayed very weak cytotoxicity belonged to the T or non T cell fraction Lymphoid cells of this patient and of a control matched for age and sex were fractionated 48 hours after this patient s first IF injection While unfractionated lymphocytes of the patient and the control contained essentially similar frequencies of E and EAC rosette forming cells the frequency of EA rosette forming cells was somewhat lower in the patient (Table I) Fractionation of the lymphocytes showed that the frequency of EA rosette forming cells in the non T cell enriched fraction of the control was 51% whereas the corresponding value for the patient was 11% (Table I)

DISCUSSION

In vitro exposure of peripheral lymphoid cells from healthy donors to preparations of human leukocyte IF augments their spontaneous cytotoxicity (6 25) Since patients at the Karolinska Hospital are treated with such IF preparations it was of interest to examine whether the spontaneous cytotoxicity of their lymphoid cells was also augmented by IF administration in vivo The spontaneous cytotoxicity of the lymphoid cells from five tumor patients was studied before and at various times after the first IF injection The spontaneous cytotoxicity of four of these patients was increased 12 hours after injection (Figs 1A 2A 3A and 5A) and remained elevated for at least another 12 hours in three (Figs 1A 3A and 5A) The increment of spontaneous cytotoxicity seemed to be preceded by a temporary reduction (Figs 1A 2A 3A and 5A)

It is not known whether the augmentation of cytotoxicity is due to a direct action of the IF preparation used or if other mechanisms are involved For instance some of the patients developed fever after the administration of IF which is a well known side effect (10 24) No relation seemed to exist however between the extent of [illegible] and augmentation of cytotoxicity The seru[illegible] 1–12 hours after an intram[illegible]

million units of IF is 10–100 units/ml (4) The fact that in vitro exposure of lymphoid cells to these IF concentrations can augment their spontaneous cytotoxicity (6 25) indicates that the increased cytotoxicity following administration of IF in vivo may be caused by a direct action of the IF preparation on the lymphoid cells This does not prove however that IF and not contaminating substances in the preparations are responsible for this effect

There may be two possible mechanisms which are not mutually exclusive for the augmentation of spontaneous cytotoxicity after injection of IF One could be by an activation of existing spontaneous killer cells the other is that IF injection changes the cellular composition of the lymphocyte population in such a way that the frequency of killer cells is increased Although the frequencies of E- EA- and EAC-rosette forming lymphocytes exhibited only minor fluctuations after IF administration in vivo (Table I) it cannot be excluded that the latter explanation may contribute to the augmented cytotoxicity The frequency of spontaneous killer cells may be so low that even a minor decrease in the frequency of another cell population for instance T-cells may be due to a manyfold increase in the number of spontaneous killer cells

For technical reasons our cell preparations were not depleted of phagocytic cells before measuring their spontaneous cytotoxicity This leaves the possibility that at least some of the effects observed are due to the cytotoxic activity of other cells than lymphocytes It has been reported that IF may activate monocytes–macrophages in such a way that they become cytotoxic or prevent proliferation of tumor cells in vitro (20 21) However we would like to mention that the augmentation of spontaneous cytotoxicity of lymphoid cells of healthy subjects occurring after treatment with purified IF in vitro is not changed to any detectable extent after depletion of phagocytic cells (unpublished results) This suggests that the cytotoxicity observed in the present study is mainly mediated by lymphocytes

One question of interest is whether injection of 3 million units of IF causes maximal augmentation of the spontaneous cytotoxicity In an attempt of answer this we examined whether the spontaneous cytotoxicity of the lymphocytes after IF injection could be further augmented by IF treatment in vitro At the time of the maximal increase of lymphocyte cytotoxicity 12 hours after the injection of IF this seemed to be the case in two patients (Figs 3B and 5B) but not in two others (Figs 1B and 2B) Our results do not permit any conclusions in this question

The lymphocytes of one of the patients exhibited very low spontaneous cytotoxicity before and after IF injection and this cytotoxicity was not augmented by IF in vitro (Fig 4A and B) One explanation could be that this patient had an abnormally low level of spontaneous killer cells This explanation is supported by the observation that the percentage of EA rosette forming cells in his non-T-cell fraction was only one fifth of that of a control (Table I) The explanation that this patient had a normal level of spontaneous killer cells but that they were lacking reactivity for Chang cells seems unlikely since cytotoxicity could not be detected using osteosarcoma cells as targets

In conclusion injections of human leukocyte IF can augment the spontaneous cytotoxicity of peripheral lymphocytes It is not known whether such an increase is of benefit for the tumor bearing patient

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Lymphocyte Subpopulations in Chronic Lymphocytic Leukemia (CLL)

Relation to the Activity of the Disease

Håkan Mellstedt, Dagny Pettersson and Goran Holm

From the Department of Medicine, Serafimerlasarettet, Stockholm, Sweden

ABSTRACT The blood lymphocytosis in CLL is caused mainly by a rise of immunoglobulin (Ig)-bearing leukemic lymphocytes. Most cells carry Fc receptors, while the percentage with receptors for human complement is very low with the present technique. The leukemic lymphocytes carry only *one* of the light chain types, which suggests a monoclonal origin. CLL patients with lymphocytes expressing κ light chains may have a more benign disease than λ-CLL. T-lymphocyte levels are high during the early course of the disease but decrease with its progression and are low in patients with 'active' disease.

Key words: Chronic lymphocytic leukemia, lymphocyte subpopulations, clinical correlation

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Malignant lymphoproliferative disorders can be classified according to the main lymphocyte subpopulation constituting the malignant cell clone. Myelomatosis, Waldenström's disease and most cases of CLL are B-lymphocyte malignancies (11, 16). Only a few cases of T-lymphocyte CLL have been reported and they seem to be associated with a poor prognosis (5). Non-Hodgkin lymphomas, on the other hand, probably emerge from different lymphocyte subpopulations, T-cells, B-cells and "null"-cells. Acute lymphocytic leukemia may be classified in a similar way. This classification can yield prognostic information (3, 4).

The aim of the present investigation was to study some lymphocyte subpopulations in CLL, with particular reference to their correlation to prognosis and activity of the disease.

STUDY POPULATION AND METHODS

Patients

A total of 37 patients admitted to the Department of Medicine, Serafimerlasarettet, Stockholm, were included. The main criteria for the diagnosis of CLL were lymph node enlargement, large amounts of mature small lymphocytes in the bone marrow together with more than 10000 lymphocytes/μl in the blood. No patient had received chemotherapy, corticosteroids or radiotherapy.

Controls

Healthy persons between 20 and 6[illegible] years of age were used as controls.

Antisera

A polyvalent antihuman Ig serum was raised in rabbits by injection of pooled human IgG (Kabi, Stockholm, Sweden). The rabbit IgG fraction was isolated by Sephadex G-200 chromatography. An aliquot of the rabbit IgG fraction was digested by pepsin. $F(ab')_2$-fragments were purified by gel filtration through Sephadex G-200. Both digested and undigested antibodies were conjugated with fluorescein isothiocyanate. The F/P molar ratio was 1:2. The antibodies had specificities for both κ and λ determinants and reacted with all Ig classes. The antisera are here referred to as polyvalent anti-Ig.

Antiserum against γ-chains was raised in rabbits by injection of pooled human IgG. The antibody containing IgG fraction from the rabbit serum was isolated and $F(ab')_2$-fragments were obtained by pepsin digestion. The antiserum was heavily absorbed by immunoadsorbents. The antigens were insolubilized by glutaraldehyde cross-linking (7) or by coupling to cyanogen bromide activated Sepharose-4B (15). The antiserum containing γ-chain antibodies was adsorbed with pooled IgM, IgD and Bence Jones κ and λ-proteins.

Antisera against μ- and δ-chains were obtained by immunizing rabbits with isolated IgM proteins from patients with Waldenström's disease and IgD-λ myeloma protein, respectively. The rabbit IgG fraction was purified by Sephadex G-200 chromatography and adsorbed with α_2-macroglobulin and pooled human IgG. The anti-μ serum was further absorbed with IgD myeloma [illegible] Bence Jones κ-chains and the anti-δ serum [illegible] Waldenström IgM protein and [illegible] digested antibodies were conjugated with [illegible] thiocyanate

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CLL = chronic l...

IFL = ...

sheep ...

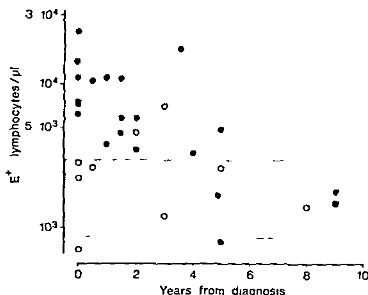


Fig 2 Correlation between total E lymphocytes in untreated CLL patients with inactive (●) or active (O) disease and time from diagnosis Shaded area indicates normal range for E⁺ lymphocytes

The blockage of CLL lymphocytes is visualized in the fluorescence microscope by the homogeneous often faint staining of all lymphocytes which is quite different from the inhomogeneous fluorescent pattern seen on normal B lymphocytes (11)

The monoclonal nature of the leukemic cells was demonstrated by the presence of one light chain type on the majority of cells in an individual patient (Table II) (18)

When the leukemic cells expressed κ-chains the malignant disease was commonly inactive suggesting a more favourable prognosis compared to emias with λ chains on the cell surface where ost patients (5/7) required active therapy This is in line with Hamblin and Hough s observation that elderly women with CLL and κ chains on the leukemic lymphocytes required no treatment (8) Furthermore it has been reported that light chain myelomas producing κ Bence Jones protein are more benign than those producing λ Bence Jones protein (1) IgD myelomas where the light chain component is of λ type in 90% are thought to have a poor prognosis (13) Moreover the M-component is IgM κ in about 80% of patients with Waldenström s disease and the prognosis is usually good (19) It is attractive to assume that B lymphoproliferative malignancies emanating from λ synthesizing cells may represent more aggressive disorders However extended studies are required to fully elucidate this problem

The results presented in Figs 1 and 2 show that there was a tendency to low E⁺ lymphocyte counts in patients with inactive disease a long time after diagnosis In most patients with an aggressive disease the E⁺ lymphocyte counts were low regardless of the time after diagnosis These findings may be analogous to those described in patients with solid tumors Reduced T lymphocyte functions have been noticed in patients with advanced cancer (6) An alternative explanation might be that active CLL with low T lymphocyte counts represents a separate variety of the disease

The increased blood E⁺ lymphocytes in patients with CLL may be explained in various ways Some leukemic B lymphocytes may have surface characteristics in common with T lymphocytes (10) Some of these cells may bind SRBC and therefore be identified erroneously as T lymphocytes In this context it should also be mentioned that CLL patients who enter complete remission after treatment with cytostatics have only slightly increased circulating B lymphocytes and normal T lymphocyte values (9 10)

Human B lymphocytes can be identified as carrying sIg and part of these cells also have complement receptors (for C3b and C3d) or receptors for IgG Normally the numbers of EAC⁺ and EA⁺-cells are of the same order of magnitude and exceed that of sIg⁺-cells In CLL however only a few EAC⁺-cells could be identified by our rosette technique using human complement which preferentially detects C3b However CLL lymphocytes seem to retain the receptor for C3d (mouse complement) and the numbers of sIg⁺ and EACd⁺-cells closely follow

each other (17) The inability to detect EACb+ cells in CLL may depend on loss of the receptor due to the malignant transformation An alternative explanation might be that the leukemic cells do not emerge from cells carrying receptors for C3b

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Familial Antithrombin III Deficiency as Pathogenesis of Deep Venous Thrombosis

Lilian Johansson Ulla Hedner and Inga Marie Nilsson

From the Coagulation Laboratory Malmö General Hospital Malmö Sweden

ABSTRACT A family including 18 members with decreased antithrombin III (AT III), measured with both a biological and an immunochemical method, is described The pattern found on crossed immunoelectrophoresis, using heparin in the agarose in the first run was normal, though the peaks were low This suggests decreased synthesis of a normal protein in the affected members AT III deficiency occurred in both the paternal and the maternal branch of the propositus Six, all belonging to the maternal branch of the above 18 persons had had at least one thromboembolic episode Some of the episodes had been precipitated by the presence or occurrence of some predisposing event or circumstance This suggests the possible occurrence of a gene making some of the maternal family members more susceptible to certain trigger factors, such as surgery, infection pregnancy and the puerperium The mode of inheritance filled all the criteria for autosomal dominant transmission Prophylactic treatment preferably oral anticoagulants and/or dextran is recommended for all persons with a low AT III concentration in any situation known to increase the predisposition to thrombosis The effect of heparin in these patients is impaired since the heparin co-factor, which is identical with AT III is lowered

Key words Venous thrombosis antithrombin III antithrombin III deficiency

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Familial thromboembolism associated with decreased antithrombin III (AT III) activity was first described by Egeberg in 1965 (6) AT III deficiency has since been described as *the cause of familial* deep venous thrombosis (DVT) by several authors (4 9 13 15 16 22) AT III has been determined with both a biological and an immunochemical method (1 3 7) The correlation between the results obtained by the two methods is as a rule high (? 9 16) However the biological method cannot be replaced by the immunochemical This was clearly exemplified by Sas et al (22) in a family in which the AT III was normal according to the immunochemical method but low when measured with the biological method Their group also showed that further information about the AT III function and protein can be obtained with the crossed immunoelectrophoresis technique

Hereditary AT III deficiency as a cause of familial DVT seems to be rare At our laboratory we have detected only 4 families with the disorder since the beginning of routine determination of AT III in the late sixties (roughly 175 primary examinations a year) This paper reports an investigation of 44 members of the largest of these families None of the examinations were performed earlier than 4 weeks after the end of an episode of DVT

METHODS

The following determinations were made platelet count platelet adhesiveness factor VIII activity factor VIII related antigen P & P (prothrombin + proconvertin + factor X) factor V fibrinogen fibrin/fibrinogen degradation products plasminogen euglobulin clot lysis time fibrinolytic activity of resuspended euglobulin precipitate of plasma on unheated fibrin plates α_2-macroglobulin inhibitors of plasminogen activation and fibrinolytic activity in superficial vein walls The procedures are described elsewhere (5 11 12 17–20 26) *Fibrinolytic response to venous occlusion* of the arms was performed according to Robertson et al (21) 95% confidence interval 310–380 mm^2 *AT III* was determined *(a) biologically* according to Abildgaard et al (3) using heat defibrinated plasma and *(b) immunochemically* according to Hedner and Nilsson (10) using the rocket method of Laurell (14) Antiserum against AT III was obtained from Behringwerke Marburg/Lahn West Germany Reference range for both methods 75–120% (c) *Crossed immunoelectrophoresis* with heparin in the agarose was performed according to Sas et al (24) but with the following modifications 10 ml of 1% agarose solution containing 16 6 U heparin/ml agarose were poured onto a glass plate (110×110 mm)

Abbreviations AT III=antithrombin III DVT=deep venous thrombosis

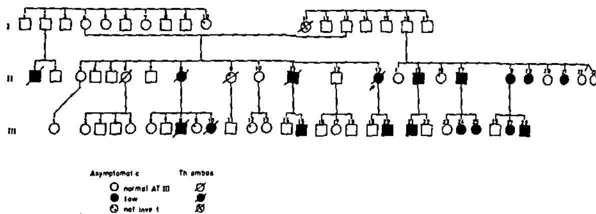


Fig 1 Pedigree of the family

After the agarose had set a well 3 mm in diameter was cut out in one corner of the plate and 5 μl of the sample was deposited into it Electrophoresis was carried out at 20 mV/plate for 4 hours at room temperature and cooled with running tap water After the first electrophoretic run the agarose gel was cut longitudinally into two parts The cut was placed 25 mm from the edge of the plate The smaller part of the agarose layer which contained the separated proteins was left untouched while the large segment was discarded and replaced by 7 5 ml of 1% agarose containing 1 5% anti AT III immune serum The plate was then turned 90° from the direction of the first run and electrophoresis was run with the plate in this direction at 10 mA overnight under the same conditions as the first run The plate was then dried and stained with Coomassie brilliant blue Normal mixed plasma was always run simultaneously for comparison with the patient s plasma When the AT III of the patient s plasma was low, the mixed plasma

a

b

c

d

Fig 2 Crossed immunoelectrophoresis in agarose gel containing 16 6 U heparin/ml agarose (*a*) Plasma from II 1 (maternal relative) (*b*) from III 21 (paternal relative) (*c* and *d*) normal plasma

Table I *Results of AT III determinations of the investigated family members according to biological (B) and immunochemical (I) methods (reference range 75–120 %)*

Family member	Born in	DVT and/or pulmonary embolism	AT III (%) B	AT III (%) I
Generation II				
1	1916	1	40	39
8	1919	>2	38	25
11	1929	1	41	34
13	1937	>2	45	38
15	1923	0	52	40
17	1927	0	49	55
18	1929	0	43	33
19	1930	0	43	46
21	1935	0	52	39
2	1920	0	129	108
3	1906	0	93	100
6	1913	1	116	86
10	1925	0	90	90
12	1932	0	124	104
14	1922	0	105	100
20	1933	0	86	120
22	1937	0	100	90
23	1937	0	92	72
Generation III				
8	1941	1	43	39
10	1955	1	50	43
15	1963	0	40	39
20	1964	0	53	35
21	1951	0	50	41
24	1965	0	43	37
25	1966	0	64	41
27	1956	0	58	50
28	1958	0	44	55
1	1932	0	82	62
2	1938	0	98	100
3	1945	0	127	124
4	1947	0		90
5	1950	0	150	124
6	1937	0	79	92
7	1939	0	90	100
11	1942	0	105	100
12	1947	0	90	68
14	1959	0	91	86
16	1960	0	98	100
17	1962	0	130	109
18	1963	0	143	100
19	1961	0	82	78
22	1953	0	82	100
23	1960	0	133	120
26	1951	0	100	100

was used both undiluted and diluted to the corresponding level of protein before the crossed immunoelectrophoresis was started

Family study (Fig 1 and Table I)

The propositus was a woman born in 1937 At 13 years of age she had had pneumonia with clinical symptoms of thrombosis of the left leg Her first pregnancy in 1961 was complicated by thrombosis of the left leg and signs of recurrent pulmonary embolism Her second child was born in 1964 pregnancy and parturition had been uncomplicated In 1974 she had apparently had spontaneous phlebographically verified thrombosis of the left leg on two occasions within about 6 weeks She was treated with heparin and after her second spell in hospital she continued treatment with Waran® (warfarin sodium) Regular follow up with Thrombotest® showed values within therapeutic ranges but although the treatment seemed to be effective a few weeks later she developed typical signs of pulmonary embolism which regressed spontaneously She continued to take Waran® and has since felt well and had no symptoms of thromboembolism Investigation revealed that AT III was low (45% biological method 37% immunochemical method) All the other coagulation factors studied were normal as were the platelets the fibrinolytic components and the fibrinolytic activity in the vessel walls

This patient had a large kindred and some relatives had had DVT Four of the 23 members of the proband s generation II besides herself had had DVT Moreover one sister died at the age of 20 years from thrombosis and pulmonary embolism in the puerperium In as many as 9 of the 18 examined members of generation II AT III was significantly decreased

All the members with a history of DVT had low AT III except a woman (II 6) who had once had DVT during pregnancy but a normal AT III level when examined about 25 years later Five of the 9 persons with low AT III were paternal cousins All of them denied any previous episode of DVT A maternal cousin (II 1) had had DVT and his AT III was abnormally low on repeated occasions Liver function tests showed nothing remarkable Consanguinity was denied

Twenty six members of generation III were examined Only 2 of them have so far had DVT at 25 and 20 years of age respectively In one of them thrombosis had apparently been spontaneous and in the other it had occurred in the puerperium In these 2 and in 7 others AT III was low in the remaining 17 members it was normal Also 4 children of II 6 (with a history of DVT but normal AT III) were examined AT III was normal in all of them Thus AT III was low in 18 of the 44 examined members of the family Of the 18 four all belonging to the maternal branch of the family had had thromboembolism on at least one occasion Repeated crossed immunoelectrophoresis of plasma from 9 members (II 1 15 17 21 III 21 24 25 27 28) with low AT III showed a normal pattern though the peaks were low compared with the mixed plasma (Fig 2) As expected crossed immunoelectrophoresis without heparin showed only one peak

The correlation between the AT III values obtained by the biological assay and those determined immunochemically was high

DISC ION

Since Egeberg (6) f
high incidence of

decreased AT III activity about 20 other families have been described This paper reports a further family Our finding that of 18 members with a significantly low AT III level 6 had previously had thrombosis at least once confirms the association between low AT III and predisposition to DVT The heredity of AT III deficiency is autosomal dominant (6 13 15 16) Our pedigree also fills all the criteria for autosomal dominant inheritance (*a*) both sexes are affected equally often (*b*) the trait appears in every generation (*c*) the trait is transmitted by an affected person to half of his children (on average) and (*d*) unaffected persons do not transmit the trait to their children (25) Maternal and paternal relatives of our proband carried the abnormal gene suggesting the recessive transmission The heredity of AT III deficiency is however doubtlessly autosomal dominant because of the rarity of the condition in the general population

AT III deficiency and DVT were more common on the mother s than the father s side Actually none of the members on the father s side had had any signs of DVT though AT III was low in 10 of them This might be explained by a modifying gene counteracting the development of DVT It might also be explained by some environmental factor predisposing the affected members of the maternal branch to thrombosis In fact the propositus had her first attack of DVT in association with pneumonia and her second in connection with childbirth Further one of the proband s sisters died from pulmonary embolism in the puerperium The only person examined who had had DVT despite a normal AT III level developed it in the puerperium Such predisposing factors were less common among the paternal members with low AT III For example 2 women with low AT III were never pregnant Another factor is age the members on the father s side were on average younger than on the mother s and therefore less predisposed to DVT Egeberg (6) found that in the family described by him those members who had a low AT III level and developed thromboses did so in connection with trauma surgery infections or pregnancy It should therefore be stressed that such factors aggravate the predisposition to thrombosis in persons with a low AT III level and that they should receive prophylactic treatment

In all the members studied by us the correlation between the results of the biological and immunochemical methods was high This means that none of them had the type of AT III deficiency described by Sas et al (23) and characterized by a normal amount of protein but with impaired function There are obviously various forms of AT III deficiency which underlines the importance of checking both the protein activity and protein related antigen

When crossed immunoelectrophoresis with heparin (16 6 U/ml agarose) was introduced for investigation of AT III by Sas et al (23) they found that 3 peaks appeared in normal plasma a large fast one and 2 small slower ones When heparin was not included in the first electrophoretic run only a single high peak could be demonstrated Examinations of our patients with decreased AT III showed a similar pattern with one high anodal peak followed by 2 lower ones All the peaks were however lower than those in normal plasma This contrasts with observations in the families investigated by Sas et al (23) and Gomperts et al (9) who found a low first peak migrating at a normal rate but a relatively larger second peak and no third peak The pattern found in the family investigated by us implies a decreased synthesis of a normal protein as the cause of the low AT III levels

Thus several of the family members had low AT III most probably due to a decreased synthesis of a normal protein The genetic pattern is that of autosomal dominant inheritance The low AT III level was very often associated with DVT Additional environmental factors known to predispose to DVT seemed however to be important contributing causes of the thromboembolic episodes It is therefore recommended that persons with a decreased AT III level should be given prophylactic treatment in any situation involving an increased risk of thrombosis such as infections surgery and other conditions requiring long bed rest Oral anticoagulants and/or dextran are recommended for such treatment since heparin does not produce the desired effect when AT III which is identical with the heparin co-factor is deficient

In acute DVT thrombolytic therapy might be considered Oral anticoagulants alone or dextran could also be given A satisfactory effect of heparin alone cannot be expected as AT III is the heparin co-factor but it might be used combined with plasma to supply a certain amount of AT III If acute DVT appears during pregnancy dextran is recommended since it does not enter the placenta nor is it teratogenic as oral anticoagulants (8)

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The Effect of the Converting Enzyme Inhibitor SQ 20 881 on Kinins, Renin–Angiotensin–Aldosterone and Catecholamines in Relation to Blood Pressure in Hypertensive Patients

Lennart Hulthén and Bernt Hökfelt

From the Department of Endocrinology Lund University Clinics Malmo General Hospital Malmo Sweden

ABSTRACT Nine sodium replete hypertensive patients with normal or high plasma renin activity (PRA) were given SQ 20 881 1 mg/kg intravenously BP fell significantly within 20 min and reached its lowest level after 60 min Blood kinins showed a minor but significant decrease after 60 and 105 min Plasma angiotensin II was markedly reduced after 15 and 60 min PRA was significantly increased after 15–105 min Plasma aldosterone was reduced at 60 min in eight patients and slightly increased in one Plasma noradrenaline increased after 15 min, whereas adrenaline decreased after 60 min These results indicate that the reduction of BP following inhibition of converting enzyme by SQ 20 881, 1 mg/kg is not related to reduced degradation of kinins but rather to decreased formation of angiotensin II Angiotensin II appears to be of importance for the maintenance of BP in sodium replete hypertensive patients with normal or high PRA

Key words Kinin renin–angiotensin–aldosterone catecholamines converting enzyme inhibition hypertension

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The angiotensin-converting enzyme is most likely identical with kininase II (7) This peptidyldipeptide hydrolase is widely distributed in the body (7) The highest activities are found in the pulmonary vascular endothelium and the brush border of the proximal renal tubule (7) As this enzyme on one hand generates angiotensin II the most potent vasoconstrictor identified (19) on the other hand degrades bradykinin the most potent vasodilator known (8) it may play an essential role in BP regulation

Recently a nonapeptide (SQ 20 881) which inhibits the conversion of angiotensin I to angiotensin II and potentiates bradykinin was identified and synthesized (10) In man SQ 20 881 reduces BP in hypertensive patients with normal or high plasma renin activity (PRA) (1 9 17)

The aim of the present study was to investigate the effects of SQ 20 881 on kinins in blood in relation to its effect on renin–angiotensin–aldosterone catecholamines and BP

PATIENTS

Six men and three women with hypertension were studied Eight were classified as WHO grade I–II and one as grade III (Table I) Plasma and urinary electrolytes endogenous creatinine clearance and urinary catecholamines were normal in all when off treatment Both i v pyelography and renal angiography were performed in seven patients only pyelography in two and only angiography in two PRA was determined in samples from both renal veins in seven patients On the basis of the information thus obtained four patients were considered to have essential and five renovascular hypertension

All patients were without antihypertensive medication for at least two weeks prior to the administration of SQ 20 881 Seven received no drugs during the study one (no 8) was given sulfonamide (Sulfapral®) for a lower urinary tract infection and another (no 9) was on long term digoxin due to previous cardiac failure

The studies were performed under metabolic ward conditions Following admission the patients were maintained on a diet containing 120 mEq of sodium and 95 mEq of potassium per day for one week prior to the administration of SQ 20 881 On day 5 when the patients were in electrolyte balance urinary aldosterone excretion was normal in four patients and high in five (Table I) urinary excretion of sodium being 99±6 (mean ± S E M) and potassium 90±4 mEq PRA as measured peripherally in the supine position in the next morning was normal in three patients and high in six (Table I)

Address for reprints L Hulthén M D Department of Endocrinology Lund University Clinics General Hospital S 214 01 Malmo Sweden

Abbreviations PRA=plasma renin activity BP=blood pressure ECG=electrocardiogram

Table I *Clinical data on the patients*

Case no	Age (y)	Sex	Hypertension Type	WHO stage	Supine PRA[a] (ng/ml/3 h)	Urinary aldosterone[b] (nmol/d)	BP (mmHg)[c] Supine	Upright
1	63	♂	Essential	I–II	1 13	36 2	145/98	145/103
2	49	♀	Essential	I–II	1 38	37 8	154/107	135/102
3	22	♀	Essential	I–II	3 83	52 0	136/103	143/106
4	46	♂	Essential	I–II	4 00	39 5	162/110	153/115
5	56	♂	Renovascular	I–II	1 97	46 1	162/99	160/101
6	64	♂	Renovascular	I–II	2 82	28 7	168/111	130/103
7	55	♂	Renovascular	I–II	4 05	80 9	171/106	158/104
8	47	♀	Renovascular	I–II	4 49	67 9	179/112	152/110
9	52	♂	Renovascular	III	10 05	112 6	162/105	155/109
Normal range					*0 5–2 0*	*8–40*		

[a] At 7 a m on day 6 [b] On day 5 [c] Measured once every hour in the supine position at 7–11 a m and in the upright position between noon and 4 p m on day 7 using a 12 5 cm wide and 34 cm long rubber cuff connected to a mercury sphygmomanometer Diastolic pressure was registered at the disappearance of the Korotkoff sounds The figures represent mean systolic and diastolic pressures

METHODS

Experimental design

For the special studies with SQ 20 881 (supplied by Dr E Rucinska Squibb Clinical Research Department Princeton New Jersey) the patients were kept in supine position from 10 p m on day 7 until 2 hours after the injection of SQ 20 881 on the next day (i e 11 00–11 40 a m) On day 8 BP was measured with an intra arterial catheter (see below) connected to an electronic transducer (EMT 746 Siemens Elema Sweden) and re corded on a Mingograph 81 (Siemens Elema) every 10 min for 20 min before and for 110 min after the injection of SQ 20 881 starting between 8 40 and 9 20 a m Heart rate was continuously recorded from the ECG SQ 20 881 1 mg/kg (10 mg/ml in destilled water) was given as an i v injection between 9 00 and 9 40 a m through a siliconized le in an antecubital vein over a period of 3 min

Blood samples for determination of kinins angiotensin II PRA aldosterone and catecholamines were collected at various intervals before and after administration of SQ 20 881 as indicated in Fig 1 The samples were drawn from a polyethene catheter (PE 160 Intramedic Clay Adams New York) inserted into the left brachial artery between 8 20 and 9 00 a m using the Seldinger technique The total amount of blood drawn from each patient was 360 ml

To inhibit surface activation of prekallikrein the catheter was immersed in 0 05% solution of Polybrene (Aldrich Europe Belgium) for at least 30 min before use (5) The reason for introducing this treatment was that in the first patient studied (no 4) we used a catheter not treated with Polybrene and found a high basal kinin concentration (1 77 ng/ml) which then decreased continually following SQ 20 881 (0 92 0 78 and 0 57 ng/ml at +15 +60 and +105 min) It seems likely that this high basal level was

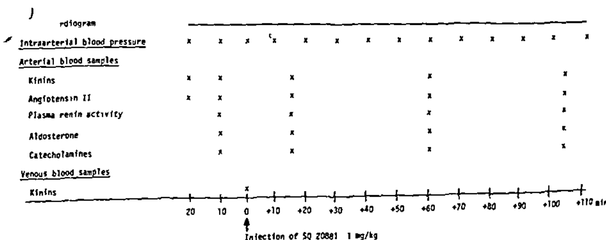


Fig 1 Experimental design on day 8 from 20 min before until 110 min after the injection of *SQ 20 881*

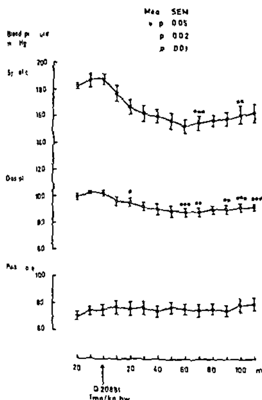


Fig 2 BP and pulse rate before and after the injection of SQ 20 881

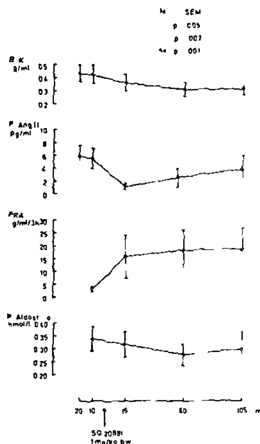


Fig 3 Blood kinins plasma angiotensin II PRA and plasma aldosterone before and after the injection of SQ 20 881

due to activation of plasma prekallikrein on the catheter Accordingly patient 4 was excluded with respect to blood kinins In the other eight patients blood kinins were also determined in samples drawn through the venous needle immediately prior to the injection of SQ 20 881 in order to detect if prekallikrein activation had occurred on the arterial catheter

Laboratory procedures

Kinins (13) angiotensin II (18) and aldosterone (16) were determined by radioimmunoassay With the kinin antiserum used kallidin showed 97% reactivity as compared to bradykinin and major degradation products of kinins less than 0.01% cross reactivity The angiotensin II antiserum showed 100% cross reactivity for C terminal hepta and hexapeptide of angiotensin II and 0 7% for angiotensin I Cross reactivity for SQ 20 881 with the antisera for kinins and angiotensin II was less than 0 01% PRA was measured radioimmunologically as the amount of angiotensin I generated during 3 hour incubation (11) A double isotope derivative technique was used for the estimation of plasma catecholamines (6)

Statistical evaluation

Statistical analysis was performed by non parametric methods Wilcoxon s two-sample test was used for paired observations and Spearman s rank correlation to calculate correlation coefficients The values are given as mean ± S E M and the level of significance is taken as $p<0\ 05$

The study was approved by the Ethical Committee at the Medical Faculty of the University of Lund Consent was given by each patient after complete description of the protocol

RESULTS

Following the injection of SQ 20 881 both systolic and diastolic BP were significantly reduced after 20 min fell to minimum values at 60 min and were still significantly reduced at 110 min (Fig 2) Heart rate did not change significantly (Fig 2)

Basal arterial concentration of kinins was 0 43± 0 06 ng/ml which was not significantly different from the venous level (0 37±0 04 ng/ml) After the injection of SQ 20 881 there was no significant change in the arterial level of kinins at 15 min but a slight decrease at 60 and 105 min ($p<0\ 05$) (Fig 3)

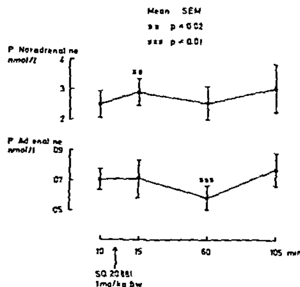


Fig 4 Plasma catecholamines before and after the injection of SQ 20 881

Angiotensin II fell in all patients after SQ 20 881 in six to well below the limit of sensitivity for our method (1 pg/ml) For graphic treatment these values were adopted as 0 5 pg/ml The level of angiotensin II was maximally reduced at 15 min ($p<0\ 01$) and still significantly reduced at 60 min ($p<0\ 01$) after SQ 20 881 (Fig 3) There were positive correlations between the basal level of angiotensin II and its decrease at 15 and 60 min ($r=0\ 96$ $p<0\ 001$ and $r=0\ 74$ $p<0\ 05$ respectively) Neither the basal level of angiotensin II nor its decrease was significantly correlated to the reduction of systolic or diastolic BP

PRA increased in all patients after SQ 20 881 ($p<0\ 01$) (Fig 3) Maximal value was recorded at 5 min in one at 60 min in three and at 105 min in five patients At 15 min PRA was positively correlated to basal PRA ($r=0\ 71$ $p<0\ 05$) There was a direct relation between basal PRA and basal angiotensin II concentration ($r=0\ 76$ $p<0\ 05$) At 15 min the increase in PRA was positively correlated to the decrease in angiotensin II ($r=0\ 68$ $p<0\ 05$) Neither basal PRA nor its absolute increase was significantly correlated to the reduction of BP There was however a positive correlation between the percentage increase in PRA and the reduction of systolic and diastolic BP ($r=0\ 70$ $p<0\ 05$)

Plasma aldosterone decreased in 8 patients and increased slightly in one at 60 min after SQ 20 881 but the change was not significant (Fig 3) There was no correlation between the basal levels of angiotensin II and aldosterone nor between the fall in these two hormones after the injection of SQ 20 881

Plasma noradrenaline concentration was significantly increased 15 min following SQ 20 881 ($p<0\ 02$) Plasma adrenaline concentration showed a significant decrease at 60 min ($p<0\ 01$) after SQ 20 881 (Fig 4)

During the two hours immediately after SQ 20 881 when the patients were supine no adverse effects were observed When rising thereafter three patients (nos 3 4 and 8) fainted and two (nos 2 and 6) felt dizzy and weak The others were ambulatory without circulatory symptoms In the first mentioned five patients there was a mean decrease of 4 pg/ml in angiotensin II and 0 13 nmol/l in aldosterone at 105 min after SQ 20 881, whereas the other four patients showed mean increases of 1 pg/ml and 0 06 nmol/l respectively It is also noteworthy that the five patients with orthostatic symptoms had a mean increase in PRA of 267% at +105 min against 981% in the others No differences between patients were noted with respect to changes in catecholamines at +105 min

DISCUSSION

Our studies show that SQ 20 881 is effective in reducing systolic and diastolic BP in sodium replete hypertensive patients with normal or high PRA thus confirming earlier reports (1 9 17)

A minor decrease in arterial kinin concentration was found after 60 min following SQ 20 881 A similar response was recently reported by Vinci et al (25) in sodium replete hypertensives given SQ 20 881 1 or 3 mg/kg Williams and Hollenberg (27) found an increase in arterial bradykinin levels in sodium deplete hypertensives following SQ 20 881 0 03 mg/kg These investigators used a different technique for the preparation of blood samples and their bradykinin concentrations were distinctly higher than ours

The basal arteriovenous difference in kinins found in our patients is very similar to that reported by Mashford and Zacest in normal men (21) suggesting that treatment of the catheter with Polybrene effectively inhibited prekallikrein activation

Reduction of renal perfusion pressure and/or renal blood flow is associated with a decrease in renal venous kinin output in man (14 15) Infusion

of SQ 20 881 in sodium replete dogs reduces renal blood flow (20) Thus the slight decrease in arterial kinin concentrations found in our studies at 60 and 105 min after SQ 20 881 could be due to the decrease in BP or in renal blood flow

The lack of an increase in kinins following SQ 20 881 in a dose which effectively inhibited conversion of angiotensin I to angiotensin II is not incompatible with the view that converting enzyme and kininase II are identical Converting enzyme from hog lung has an affinity for bradykinin which is 35 times higher than for angiotensin I (K_m 0 85×10^{-6} and 30×10^{-6} M/l respectively) (4) and purified converting enzyme from human lung has a specific activity for bradykinin which is 14 times higher than that for angiotensin I (22) This indicates that a higher dose of SQ 20 881 is required to inhibit kinin degradation than to inhibit angiotensin conversion It is not likely that other enzymes than a peptidyldipeptide hydrolase are of major importance for the pulmonary degradation of kinins When H Phe[8] bradykinin was infused into the pulmonary artery in four patients undergoing cardiac catheterization 60–70% of the administered radioactivity was recovered as ^{3}H Phe Arg in the brachial artery after 5–18 sec (7)

The observed time lag between the reduction of plasma angiotensin II concentration and BP is similar in length to that observed in rabbits when discontinuing long term infusions of small amounts of angiotensin II (3)

The positive correlation between the increase in PRA and the decrease in angiotensin II at 15 min after the injection of SQ 20 881 supports the view that angiotensin II exerts a negative feedback on renin release (2)

The increase in plasma noradrenaline following SQ 20 881 indicating increased sympathetic activity was probably due to the fall in BP (26) Studies in man have shown that central sympathetic activity is enhanced by an injection of angiotensin II (24) Our finding of increased noradrenaline at 60 min after SQ 20 881 when angiotensin II was maximally reduced suggests however that angiotensin II is only of minor importance for the regulation of sympathetic activity

Under experimental conditions both angiotensin I and angiotensin II are active in stimulating the release of catecholamines from the adrenal medulla (23) Thus it seems not likely that the inhibition of angiotensin conversion played any role for the decrease in plasma adrenaline at 60 min following SQ 20 881

The orthostatic reactions in five of our patients were probably mainly due to the prolonged decrease in circulating angiotensin II The distinctly lower percentage increase in PRA in these five patients than in the other four was likely insufficient to overcome the converting enzyme inhibition

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Clinical Aspects on 64 Cases of Juvenile and Adult Listeriosis in Sweden

Sture Larsson Stig Cronberg and Sten Winblad

From the Departments of Infectious Diseases and Bacteriology University of Lund Malmo General Hospital Malmo Sweden

ABSTRACT In 1958–74 altogether 64 cases of bacteriologically verified infections of Listeria monocytogenes were diagnosed in Sweden in children, aged more than 27 days, and in adults Immunosuppression predisposed to the disease Thus, many patients had co-existing disorders such as leukemia and alcoholism Sixteen patients had been treated with corticosteroids, which were combined with cytostatic drugs in nine Meningoencephalitis was diagnosed in 52 patients and was fatal in 16 The clinical symptoms did not differ from those in purulent meningitis caused by other bacteria In the cerebrospinal fluid the cellular response was dominated by polymorphonuclear cells in 29 patients and by mononuclear cells in 20 Ten patients had septicemia, which was fatal in four Clinical symptoms were dominated by chills, high fever and general prostration One patient had pleurisy and one an abscess of the neck both recovered Serotypes 1 and 4b prevailed and were equally common Many patients developed raised antibody titers in both the O agglutination test and the complement fixation test The titers were often not positive until after a month Moderate granulocytosis was the rule and monocytosis was rarely seen Ampicillin alone or combined with an aminoglycoside seemed to be the drug of choice in the treatment of listeriosis An alternative drug was tetracycline Most deaths occurred within six days of onset of the illness Early diagnosis and treatment were imperative Most patients recovered and serious sequelae were rare

Key words Listeria monocytogenes immunosuppression meningoencephalitis septicemia ampicillin aminoglycoside

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Listeriosis is an infection affecting mainly newborns pregnant women and immunocompromised patients (2 5 25) Bojsen Moller reported 59 cases of listeriosis in Denmark (2) Most other reports of listeriosis concern only a small number of cases (1 3 -4 6 8 9 10 14 16 21 22 24 29 30)

In Sweden the first human case was diagnosed in 1958 (21) The disease was made notifiable in 1960 *In 1958–74 110 cases of listeriosis were registered* in Sweden (17) In other publications we report the epidemiological aspects (17) the morbid anatomy (to be published) and clinical findings in 46 pregnant women and their neonates (19) Clinical aspects in 64 cases of listeriosis in juveniles and adults will be reported below

STUDY POPULATION AND METHODS

Patients

The patient series consisted of all 64 cases of listeriosis that were diagnosed in Sweden in children more than 27 days of age and in adults in 1958–74 The patients were admitted to hospitals in various parts of Sweden The following departments diagnosed cases of listeriosis Departments of Infectious Diseases 44 Departments of Internal Medicine 12 Departments of Renal Diseases 4 Departments of Pediatrics 2 and Departments of Surgery 2 cases

The diagnosis was verified in all cases by isolation of Listeria monocytogenes (L m) The micro-organism was cultured from the cerebrospinal fluid (CSF) blood pleural effusion abscess and in post mortem material from the central nervous system and the liver

Clinical information was obtained from a study of all patients records and was sometimes supplemented by direct information from the patients and their relatives or from members of the hospital staff

Part of this material has been published earlier as case reports (3 4 8 29)

Address for reprints S Larsson M D Department of Infectious Diseases General Hospital S 214 01 Malmö Sweden

Abbreviations L m =Listeria monocytogenes CSF=cerebrospinal fluid O aggl=O-agglutination CF=complement fixation

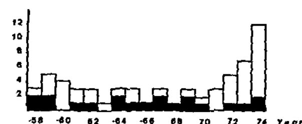


Fig 1 Annual incidence and mortality of listeriosis in Sweden 1958–74 Children and adults 64 cases 44 alive (□) 20 dead (■)



Fig 2 Incidence and mortality of listeriosis in different age groups in Sweden 1958–74 Children and adults 64 cases 34 males 30 females 44 alive (□) 20 dead (■)

Bacteriology

The isolated strains were diagnosed in the Laboratories of Bacteriology in Borås Falun Goteborg Karlstad Kristianstad Linkoping Lund Malmo Norrkoping Stockholm Sundsvall and Uppsala Forty four strains were sent to Malmo as a reference laboratory for identification and control

The technique used for bacteriological culture and diagnosis of L m has been described elsewhere (17) The strains were serotyped by slide agglutination with adsorbed O-sera prepared according to Seeliger (25) Three strains were serotyped by Seeliger Wurzburg Germany and three by Kampelmacher Bilthoven the Netherlands

Serology

Antibodies against L m were assayed according to Winblad (17 31)

RESULTS

Co-existing disorders and immunosuppressive treatment

The annual incidence of listeriosis is seen in Fig 1 It was diagnosed in 64 cases Most of them were 40–70 years old (Fig 2) Many had co-existing disorders mostly leukemia and alcoholism (Table I)

Table I *Co-existing disorders among 64 children and adults with listeriosis in Sweden 1958–74*

	No of cases		No of pats treated with	
	Total	Fatal	Corticosteroids	Cytostatic drugs
Leukemia*	8	5	7	4
Alcoholism	8	2		
Kidney transplant	5	1	5	5
Dialysis treatment	1	1	1	
Diabetes mellitus	3	1	1	
Mb Cushing	1		1	
Hypothyrosis	1			
Adipositas	1	1		
Rheumatoid arthritis	1	1		
Malignant lesions	2	2		
Ulcerative colitis	1	1	1	
Tbc pulm sequelae	1			
Benign hypertension	1	1		
Heart disease	8	1		
Total	42	17	16	9
No obvious co-existing disorders	22	3		

* Five patients had chronic lymphatic leukemia

Table II *Laboratory findings*

	No of cases	Median	Range
Findings in blood			
WBC/l on admission	47	11 5×10⁹	3 8–24 2×10⁹
Differential count (%) on admission*			
Granulocytes	41	80	46–92
Lymphocytes	41	15	5–50
Monocytes	41	4	0–14
Maximal ESR (mm/h)	56	60	9–162
Findings in cases of meningoencephalitis in primary CSF			
Total no of cells/l	51	550×10⁶	5–6320×10⁶
Polymorphonuclear cells/l	51	342×10⁶	2–4 320×10⁶
Mononuclear cells/l	51	240×10⁶	0–2 000×10⁶
Protein (g/l)	43	1 40	0 47–4 90

Patients with leukemia or receiving cytostatics were excluded

One diabetic patient also had rheumatoid arthritis Necropsy revealed gastric carcinoma in one patient and renal carcinoma in another Three elderly women were being treated for heart failure four had ischemic heart disease and one had rheumatic valvular heart disease Sixteen patients had been treated with corticosteroids nine of them in combination with cytostatics

Clinical diagnoses

Meningoencephalitis was the predominant clinical manifestation It was diagnosed in 52 patients In all of them the CSF gave growth of L m and in seven of them L m was present also in the blood In one patient the infection was discovered first at necropsy All eight alcoholics and all five with renal grafts belonged to this group

The primary CSF contained an increased number of both polymorphonuclear and mononuclear cells (Table II) Polymorphonuclear cells dominated in 29 patients and mononuclear cells in 20 Protein was much increased in the CSF (Table II) The concentration of glucose in CSF was more than 50% lower than in the blood in 20 patients and 50% lower in one In six patients glucose in the CSF was not decreased

The clinical symptoms were those typical of purulent meningitis The majority of the patients fell ill with severe headache stiffness of the neck and high fever (39–40°C) At least 28 patients complained of nausea and vomiting Ten patients turned deeply unconscious and six of them died Four of the ten patients were unconscious already on admission and three of them died Another 35 patients were stuporous and ten of them died whereas seven patients were quite conscious Attacks of convulsions occurred in 15 patients eight of whom died Seven patients had hallucinations and all survived

The number of white cells in the CSF did not differ between fatal and non fatal cases (rank sum test $p>0.05$) Out of nine patients who had $\leq 200\times10^6$ cells/l in the CSF three died The interval between the onset of illness and lumbar puncture was equal (median 3 days) to that in the other patients (median 2 5 days) but seven of these nine patients were immunodepressed In four out of six analysed patients in this group of nine patients glucose in the CSF was not decreased Nevertheless the CSF protein was increased in all of them (median 1 62 g/l)

Septicemia was diagnosed by blood culture in ten patients Three of them had leukemia one was receiving regular hemodialysis and one had ulcerative colitis Clinical symptoms indicated septicemia with chills and high fever Two patients vomited and had diarrhea

Pleurisy was diagnosed in a 72 year-old man with leukemia who fell ill with fever chest pain and respiratory distress Culture of the pleural effusion gave growth of L m He was cured by pleural drainage and treatment with antibiotics

Abscess of the neck was observed in an 81 year old diabetic woman She was admitted to hospital because of a 3 week-old expanding tumor of the neck She had no fever Culture of material from the

Table III *Distribution of maximal antibody titers (%) in children and adults with listeriosis and registered blood donors*

	Total no of subj	O-aggl pos (>1/40)	CF pos (>1/10)	O-aggl pos CF pos
Blood donors 1971–75	253	8	4	1 6
Blood donors 1977	100	9	4	2
Pats with listeriosis	35	49	40	26

abscess gave growth of L m She was cured by incision and drainage of the abscess without chemotherapy

Onset of illness

In all patients except two the disease started suddenly

A 68 year-old man with leukemia fell ill with headache and tiredness in July 1969 Later he felt dizzy and repeated attacks of chills supervened He was admitted to hospital in Dec that year His temperature was high and treatment *was started with tetracycline which controlled the fever* After 4 days he developed exanthema and treatment was discontinued At the end of Dec the fever recurred and chloramphenicol was given orally He deteriorated tetraplegia supervened and he died on Jan 9 1970 Necropsy revealed widespread cerebrospinal listeriosis with a large abscess in the left cerebral hemisphere as well as a large number of microscopical abscesses in the cervical medulla

A 62 year-old alcoholic man was admitted to the hospital in Feb 1971 About 12 days before he had had headache followed later by dizziness and vomiting He also had repeated attacks of pallor and confusion On admis*sion he had high fever and was confused Lumbar puncture* revealed meningitis caused by L m He was treated and survived

erotypes of L m

Serotype 1 was diagnosed in 24 cases and serotype 4b in 25 cases Two patients had serotype 2 The remaining cases were not serotyped Clinical symptoms and mortality were not related to serotypes (17)

Serology

Serological analysis was done in 35 patients (Table III) The frequency of cases with a positive titer in the O agglutination test (O aggl) combined with a positive titer in the complement fixation test (CF) (O aggl >1 40 CF >1 10) in the patients was significantly higher than in two series of blood donors ($p<0.001$) Out of 35 patients 22 had positive O aggl and/or positive CF Eleven were posi*tive within 14 days and 17 within one month* In I patients the titer was never positive In three of them serological analysis was done only 4 9 and I days respectively after onset of illness Another four patients were immunocompromised In six patients no explanation was found

Laboratory findings

Hematological analysis revealed moderate granulocytosis (Table II) Five patients out of 4 had monocytosis Maximal ESR was moderately high It reached a maximum at the end of the first week after onset In six patients the immunoglobulins were assayed in Malmö by agar electrophoresis according to Laurell (13 20) (Table IV) One patient showed signs of hypogammaglobulinemia

Antimicrobial treatment

Since the patients were treated in different hospitals with several drugs administered together or in succession evaluation of the effect of chemotherapy was difficult and must be considered with greatest caution Benzyl penicillin combined with sulphonamide and/or chloramphenicol was successful in 14 cases this treatment failed in one case Ampicillin alone was effective in six cases and combined with other drugs in a further nine cases it failed in four cases In nine cases *tetracycline* was

Table IV *Immunoglobulins (g/l) in 6 cases of listeriosis assayed by agar electrophoresis*

Pat no	IgG	IgA	IgM
1	15	2 1	1 7
2	10	2 2	2 3
3	5	1 2	1 5
4	17	2 7	0 9
5	14	1 8	
6	18	0 8	2 9
Reference range	7–15	1 0–3 0	0 3–1 8

also effective alone or combined with other drugs it failed in three cases In 40 successfully treated cases the treatment was continued for a median of 26 days Three patients were successfully treated for only 8–10 days

Outcome

Six patients with septicemia 34 with meningoencephalitis and those with pleurisy and abscess of the neck recovered completely Most of the patients left hospital within 10 weeks In two patients who had undergone renal graft transplantation and who are presented below the infection was not completely eradicated by the initial treatment

A 34-year-old man with septicemia He was treated parenterally for four days with cephalothin followed by cephalexine orally The fever subsided after a week but two days later he deteriorated with high fever and loss of consciousness L m was found in both CSF and blood He was then successfully treated with co-trimoxazole for seven weeks

A 24-year-old woman with meningoencephalitis She was treated parenterally with benzyl penicillin combined with a sulphonamide for three days followed by chloramphenicol parenterally (1 g a day) combined with oral doxycycline for 13 days followed by ampicillin parenterally (1 g a day) for eight days Three months later an abscess developed in the buttock It yielded growth of Staphylococcus aureus but simultaneously L m of the same serotype as before was cultured from the blood She was then successfully treated orally for ten days with oxacillin combined with tetracycline

Twenty patients eight men and 12 women died most of them within six days of onset of the illness Sixteen of them had meningoencephalitis (mortality 31%) and four had septicemia (mortality 40%) Co-existing disorders often decided the outcome and were present in 17 of the fatal cases Out of 22 previously healthy people only three died (Table I)

Out of 36 patients surviving meningoencephalitis three developed hemiparesis one of them combined with pharyngeal paresis Two of these patients recovered completely The third had a cerebral hemorrhage which was drained by surgery but the paresis persisted Two patients got paresis of the abducens nerve one patient nystagmus and one impairment of hearing and tremor All these patients recovered completely One patient developed numbness and impaired sensibility in half of the face These complaints were still persistent at re-examination after a year

DISCUSSION

Listeria monocytogenes is a widespread microorganism to which most of us are exposed some time in life (17) For healthy people it is mostly a mild pathogen but in compromised patients it is far more dangerous (26) As in other publications (2 26) patients with neoplasms especially of lymphatic tissue diabetes mellitus alcoholism and those receiving immunosuppressive treatment dominated our series As in other reports (15 27) meningoencephalitis was the most frequent clinical form of infection Pleurisy and pneumonia are rare (25) However L m may be easily overlooked in culture of specimens from the respiratory tract Also infection of the lymph nodes is unusual but may occur in elderly people (25)

The clinical symptoms in meningoencephalitis did not differ from those of other forms of purulent meningitis Mononuclear cells often dominated the primary CSF suggesting tuberculosis fungi or virus However the definite microbiological diagnosis must be based on culture A low cellular response in the CSF was occasionally seen in immunocompromised patients but did not indicate a bad prognosis Seizures of convulsion worsened the prognosis but this was not the case with hallucinations Patients with septicemia also lacked specific symptoms indicating listeriosis

A combination of positive O aggl and positive CF strongly suggested infection since only 1–2% of normal blood donors had this combination To detect all patients developing positive titers it was necessary to follow the titers for at least one month Certain patients did not develop positive titers Sometimes the reason might be that they were immunosuppressed Nevertheless the titers of some previously healthy patients remained negative despite clinical listeriosis

As in earlier investigations (2 5 25) a moderate granulocytosis was found and only occasionally monocytosis Hypogammaglobulinemia did not seem to predispose to listeriosis Cellular immunity seems to be more important than humoral factors for the resistance to listeriosis (5 11)

The effect of antimicrobial treatment was difficult to evaluate Ampicillin was effective in many cases Iwarson and Svensson (12) showed that a daily dosage of 12–20 g ampicillin gave sufficient concentrations in CSF also after 5 days of treatment In vitro studies suggested that a combination of ampicillin and gentamicin should be used (7) In patients al

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Interaction by Cholestyramine on the Uptake of Hydrocortisone in the Gastrointestinal Tract

C Johansson U Adamsson U Sterner and T Lindsten

From the Departments of Medicine and Endocrinology Karolinska Hospital Stockholm Sweden

BSTRACT An absolute reduction of the plasma ortisol levels and a delay of the peak concentrations ere recorded in 10 healthy subjects when a bile questering anionic exchange resin cholestyramine, as given prior to a single oral hydrocortisone dose dicating that *the resin interferes* with the uptake of neutral sterol in the human gastrointestinal tract he possibility of a direct binding of drug to resin *supported* by the affinity of hydrocortisone to olestyramine in vitro which was uninfluenced by e presence of sodium taurocholate Cholestyramine gnificantly delayed the gastric emptying of a glucose lution, indicating that the resin not only decreases t also delays hydrocortisone absorption Careful pervision is *recommended* when treatment with olestyramine is given concomitant to neutral sterol ugs

olestyramine was introduced in the treatment of unitus *in* biliary cirrhosis (3 4 6 17) and of percholesterolaemia (1 17) The anionic ex nge resin increases the faecal elimination of bile ds by binding them in the small intestine thereby venting their ileal reabsorption (11 12) After demonstration that perfused bile acids block the transport in the human colon (18) the resin been used *to control* diarrhoeas after distal ileal ections (13) Successful symptomatic treatment also been reported of the diarrhoeas after va tomy (5) of chronic diarrhoea of unknown origin and of bile reflux gastritis *after gastric* resec ns (21) Despite the more extensive use of lestyramine only few data exist on its interfer e with the uptake of other sterol compounds and gs *in* the human gastrointestinal tract (2 7 8) ng hydrocortisone as test substance the present dy was undertaken to examine if such interfer e could be demonstrated and if so to analyse possible mechanisms involved

METHODS

Human experiments

Plasma levels of cortisol after 50 mg oral hydrocortisone (Hydrocortal® Organon 20 mg) were determined on two occasions in 10 healthy subjects with and without prior intake of 4 g cholestyramine (Questran® Bristol 4 g) in a glass of tap water The order of the experiments was randomized The effect of 8 g cholestyramine was examined on a third occasion in two subjects The experiments were started at 12 noon one hour after a light meal Blood was sampled every 40th min during 200 min The plasma concentration of cortisol was determined according to de Moor et al (20)

Gastric emptying of a 300 ml glucose solution (15 g/100 ml) was determined in 5 healthy subjects on two occasions with and without prior intake of 8 g cholestyramine The technique described by Hunt and Spurell (14) was used Withdrawal and washout of the gastric content was done 40 min after intake of the glucose solution which contained polyethylene glycol 4000 (PEG 30 mg/100 ml) as a meal marker and vitamin B_{12} to indicate a complete wash out The recovered volumes were analysed for concentrations of PEG (15) The total amount of PEG recovered equals the amount remaining in the stomach at 40 min from meal intake

In vitro experiments

Hydrocortisone was dissolved in 0 15 M phosphate buffer adjusted to pH 6 8 Two stock solutions were prepared with a hydrocortisone content of 50 mg (saturated solution A) and 25 mg (unsaturated solution B) per 100 ml Increasing amounts of cholestyramine were added to 20 ml samples from the stock solutions (Table I) Prior to the addition of cholestyramine some samples were equilibrated with increasing amounts of sodium taurocholate (purissimum US Biochemical Corp) All samples were shaken for 20 min at 250°C and centrifuged at 2 500 J for 5 min The supernatants were withdrawn and analysed for concentrations of cortisol

Statistical methods

Means ± S E M are given The integrated area of the time concentration curve above the basal plasma cortisol level was calculated for each experiment and expressed in μmol min l⁻¹ Significance tests were made on paired differences according to Snedecor (22)

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Combination Chemotherapy in the Management of Superior Vena Caval Obstruction in Small-Cell Anaplastic Carcinoma of the Lung

Per Dombernowsky and Heine H Hansen

From the Department of Chemotherapy R The Finsen Institute and the Department of Medicine CT Bispebjerg Hospital Copenhagen Denmark

BSTRACT Among 225 consecutive patients with nall-cell anaplastic bronchogenic carcinoma, 26 1 5%) had superior vena caval obstruction when ie malignancy was diagnosed Of these 26 patients a onsecutive series of 22 were treated initially with ombination chemotherapy (cyclophosphamide iethotrexate and CCNU, in some cases combined ith vincristine) alone and in all these cases resolu on of the syndrome was prompt within a median of days In no case were symptoms increased tran ently by the treatment No difference in response ate was observed between the histologic subtypes of mall-cell anaplastic bronchogenic carcinoma ac ording to the WHO classification Combination hemotherapy alone is an effective treatment of iperior vena caval obstruction in patients with nall-cell anaplastic bronchogenic carcinoma

ey words Small-cell carcinoma of the lung superior ena caval obstruction combination chemotherapy

cta Med Scand 204 513 1978

Small-cell anaplastic carcinoma is together with quamous-cell carcinoma the most frequent istologic type of bronchogenic carcinoma as ociated with superior vena caval obstruction (3 2 17 21) Traditionally malignant superior vena aval obstruction has been considered an oncologic emergency indicating the use of radiotherapy (3 12 16 17 20 21)

In recent years additional information obtained from staging procedures such as bone marrow ex imination and peritoneoscopy with liver biopsy has revealed that the majority of patients with small-cell anaplastic carcinoma present with extra thoracic metastatic disease (4 11) Accordingly systemic therapy has been used increasingly as the principal treatment either alone or combined with radiotherapy resulting in objective tumor response in more than 75% of the patients and also in a significant prolongation of survival compared with no treatment or radiotherapy alone (1 8)

In a series of patients with small-cell anaplastic carcinoma we have used combination chemother apy as the initial and main therapy (9 10) The results of this treatment have been evaluated speci fically in patients presenting with superior vena caval obstruction

PATIENTS AND METHODS

A total of 225 consecutive patients with biopsy proven small-cell anaplastic carcinoma of the lung seen at Bispe bjerg Hospital and the Finsen Institute from May 1973 to April 1976 are included in the evaluation

Superior vena caval obstruction was diagnosed in the presence of elevated venous pressure in the arms dilated collateral veins over the neck and upper part of the trunk and usually respiratory distress in the absence of conges tive heart failure (16)

The diagnosis of small-cell anaplastic carcinoma was established on material obtained by bronchial biopsy mediastinoscopy or lymph node biopsy The histologic subtyping was performed according to the WHO classifi cation (14)

All patients underwent routinely the following pre treatment staging procedures clinical examination chest X ray unilateral bone marrow examination from the pos terior iliac crest and if the clinical condition permitted peritoneoscopy with liver biopsy (4 11) Biopsies or fine needle aspirations from suspected lymph nodes and cutaneous infiltrates bone scans and brain scans were performed when clinically indicated Based on the staging procedures the patients were classified as having either 'localized' disease (i e no clinically demonstrable dis ease outside one lung plus involvement of mediastinal and

Address for reprints P Dombernowsky M D Chemo therapy Department R The Finsen Institute 49 Strand boulevarden DK 2100 Copenhagen Denmark

supraclavicular lymph nodes) or advanced ("extensive") disease

The patients were managed according to one of two study protocols depending on disease extension (9 10) Patients staged as having localized disease received chemotherapy consisting of CCNU (1,3-chloroethyl 1-cyclohexyl nitrosourea NSC 79037) 70 mg/m² p o cyclophosphamide 700 mg/m² i v every 4 weeks plus *methotrexate 20 mg/m² p o on days 18 and 21 in each* cycle and radiotherapy The latter was planned to start 6 weeks after the initiation of chemotheraphy and consisted of megavoltage irradiation through parallel opposing chest ports to the tumor and mediastinum plus both supraclavicular regions In addition half of the patients *received radiotheraphy to the retroperitoneal lymph* nodes adrenal glands and brain The radiotherapy was delivered as 200 rad per day in 20 fractions over 6 weeks at 2 week intervals beginning 6 weeks after the start of chemotherapy Patients with extensive disease were treated with chemotherapy alone The chemotherapy in*cluded the same three drugs in dosages as stated above or* the same three drugs plus vincristine 1 3 mg/m² weekly for the first 4 weeks and thereafter every 4 weeks The chemotherapy was continued for 18 months or until progression of disease

Anticoagulants diuretics and steroids were not given *routinely to patients presenting with superior vena caval* obstruction

RESULTS

Among the 225 patients with small-cell anaplastic bronchogenic carcinoma seen in the period 26 (11 5%) presented at the time of diagnosis with characteristic superior vena caval obstruction This group comprised 18 men and 8 women and their median age was 58 years (range 49–67)

The primary tumor was located on the right side in 24 and on the left in 2 patients Chest X ray vealed mediastinal involvement in all cases

The median length of the period from the initial linical signs of superior vena caval obstruction to the institution of treatment was 14 days (range 7–75) Localized disease alone was present in 13 patients and advanced disease in 13 (with involvement of the liver 8 peripheral lymph nodes 5 bone marrow 3 skin 1 and of both main bronchi 3)

Among the 26 patients presenting with superior vena caval obstruction the first 4 were treated initially with chemotherapy simultaneously while the next 22 consecutive patients were treated initially with chemotherapy alone Of the former group 3 responded with complete disappearance of the superior vena caval obstruction (after 10 13 and 20 days) while one patient died with progression of the disease and pancytopenia 10 days after initiatio of the treatment In the latter group all responde with partial disappearance of the superior ven caval obstruction after a median period of 7 day (range 3–27) while complete disappearance of th obstruction was noted in 21 patients after a media of 14 days (range 5–41) One of these 22 patien died from an esophagomediastinal fistula 18 day after the start of chemotherapy

Among the 26 patients remission of bot superior vena caval obstruction and measurabl disease was seen in 21 (81%) with a median dura tion of remission of 190 days (range 34–1202+) Overall median survival time for all 26 patients wa 228 days (range 10–1209) and one patient is sti alive without signs of recurrent disease (1209 day after the initiation of treatment)

In 4 of 15 patients (27%) primarily treated wit chemotherapy and radiotherapy superior ven caval obstruction recurred (on days 174 224 25 and 316) with the disease running a fatal cours after a median period of 46 days (range 31–135) as further treatment proved ineffective

Among 11 patients with extensive disease initially treated with chemotherapy alone superior vena caval obstruction developed later in 4 (on days 65 99 187 and 203) After irradiation the superior vena caval syndrome disappeared in all 4 patients but they died after a median period of 85 days (range 47–101)

Of the patients with superior vena caval obstruc tion 7 had small-cell carcinoma of the fusiform type (WHO II 1) 5 had lymphocyte like (WHO II 2) and 6 polygonal cell type (WHO II 3) while sub*classification was not possible in 8 cases* No differ ence in response rate or survival was observed be tween the histologic subtypes

Toxicity

In patients initially treated with chemotherapy alone hematologic toxicity was modest the leukocyte nadir being a median count of 2800 leukocytes/μl (range 1100–5300) Of the 4 patients treated with both chemotherapy and irradiation one died on day 10 from severe leukopenia while the other 3 had a nadir of leukocytes of 1900–2800 μl No other significant toxicity developed during the treatment

The superior vena caval obstruction was not transiently increased by the treatment in any of the patients

DISCUSSION

the very early cases of superior vena caval obstruction reported in the literature 30–40% were secondary to benign diseases such as tuberculous mediastinitis and aneurysm associated with syphilis 2) More recent summaries indicate that cancer accounts for more than 95% of the cases of superior vena caval obstruction (16) Among malignant diseases bronchogenic carcinoma especially located the right side represents more than 80% and lymphomas and metastatic cancer the remaining)% (3 12 16 17 20)

With regard to histologic type of lung cancer the two most frequent types causing superior vena caval obstruction are small-cell anaplastic bronchogenic carcinoma followed by squamous cell carcinoma (3 5 12 17 20 21) This phenomenon is in accordance with the very early spread to the mediastinal lymph nodes as demonstrated by mediastinoscopy in small-cell anaplastic carcinoma 7) The fact that this tumor type at least as evaluated by chest X ray has a much higher tendency to be located centrally in the lung than adenocarcinoma and large-cell anaplastic carcinoma also supports this view (2)

In the treatment of superior vena caval obstruction due to malignant disease surgery has generally been of little value and radiotherapy has been the treatment of choice (12 16) Rubin et al (20) observed that radiotherapy given initially in high daily doses (400 rad) resulted in more rapid disappearance of the syndrome than did smaller daily doses and subsequent studies have confirmed this finding (3 19) Furthermore the combination of nitrogen mustard in a single dose and radiotherapy was not superior to radiotherapy alone in a controlled study of patients with superior vena caval obstruction caused mainly by lung tumors of all cell types (15) In another controlled study the combination of radiotherapy and adjuvant anticoagulation was superior to radiotherapy alone as assessed by both survival and time to clinical improvement (5) The patients in the latter investigation had mainly bronchogenic carcinoma but the cell type was not stated and the differences were not statistically significant

With regard to the clinical response to radiotherapy Howard (12) observed this to occur in 86% of 253 patients with superior vena caval obstruction secondary to bronchogenic carcinoma The response rate was independent of histologic cell type but the total dose of irradiation was a major factor and doses of more than 3 000 rad were necessary to obtain this response rate In addition the recurrence rate was lower with this dose of irradiation With chemotherapy alone Kane et al (13) obtained response of superior vena caval obstruction in all their 8 patients with small-cell anaplastic bronchogenic carcinoma—a result comparable to ours in 22 patients

In our series 11% of the patients presented with or developed superior vena caval obstruction before the start of treatment The syndrome was of recent onset (2–4 weeks) and was the major presenting complaint in these patients The rapid onset of symptoms and the rapid improvement are consistent with the high growth rate of small cell anaplastic carcinoma and fast responsiveness to combination chemotherapy as compared with the other histologic types of lung cancer (6) The rapidity of improvement and the concurrent roentgenographic and clinical tumor shrinkage strongly suggest that a direct effect on the tumor is the principal cause of the syndrome s disappearance However it appears from other studies in which phlebography and venous pressure measurements have been applied and from necropsy studies that the obstruction persists in a high percentage even though the clinical signs of the superior vena caval obstruction disappear probably because of the development of collateral venous drainage (12 21)

The 26 patients with superior vena caval obstruction in the present study were part of two prospective randomized studies of 225 consecutive patients with small-cell anaplastic bronchogenic carcinoma Patients with localized disease (10) were randomized to combination chemotherapy (cyclophosphamide CCNU and metrotrexate) plus local radiotherapy (tumor and mediastinum) or extensive radiotherapy (tumor and mediastinum upper abdominal lymph nodes adrenals and cerebrum) Patients with extensive disease (9) received either the same three drugs as above or the same three drugs plus vincristine With regard to localized disease no significant superiority of extensive radiotherapy compared with local radiotherapy was observed while in patients with extensive disease the addition of vincristine resulted in significant superiority both with regard to survival and duration of response No such significant difference was observed between the 26 patients with superior vena caval obstruction and all patients included in the two

randomized studies (9 10) This indicates that the presence of superior vena caval obstruction does not necessarily indicate a particularly poor prognosis

Our study was not designed to compare radiotherapy and combination chemotherapy in a randomized fashion but the data strongly indicate that chemotherapy is efficacious in the management of superior vena caval obstruction caused by small-cell anaplastic bronchogenic carcinoma In addition the pattern with local relapses presenting in an equal number of patients treated with and without radiotherapy provides support for this view The demonstrated effectiveness of systemic treatment using chemotherapy makes it necessary to reconsider the common use of radiotherapy in small-cell anaplastic carcinoma because radiotherapy to some extent compromises more intensive chemotherapy (Hansen personal communication) and because radiotherapy does not prolong median survival (18) At present several controlled clinical trials are dealing with this question and a definite answer should soon be at hand

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Elevation of High-Density Lipoprotein in Epileptic Patients Treated with Phenytoin

Esko A N kkila Markku Kaste Christian Ehnholm and Jorma Viikari

From the Th rd Depa tment of Med c ne the Department of Neurology and the Department of Serology and Bacter ology Un ers ty of Hels nk Hels nki and the Department of Med c ne Un ers y of Turku Turku F nland

ABSTRACT Previous observations have shown that serum alpha (h gh-density) lipoprotein (HDL) cholesterol is increased by some agents which also act as liver microsomal inducers Against this background we measured the serum HDL and other lipoprotein and apolipoprotein A levels in 28 epileptic pat ents who received phenytoin as the only med cation In comparison with healthy controls of similar age and sex the phenytoin users had significantly higher HDL cholesterol ($p<0\ 001$) and apolipoprote n A I ($p<0\ 01$) levels Highest values of both parameters were found in patients whose serum phenytoin concentration was within the therapeutic range HDL cholesterol levels were above the control mean +2 S D in 43% of the phenyto n users Hypertriglyceridem a was more common among male phenyto n users than in control males (33 vs 16%) It is suggested that phenytoin increases the secretion of nascent HDL particles (and probably also that of VLDL) by the liver and that this could be associated with the induction of hepatic microsomes Since HDL is inversely related to risk of coronary heart disease the observed increase of this lipoprotein may be an example of a beneficial side-effect of a drug

Key words Cholesterol triglyceride h gh dens ty l poprote n apoprote n A I apoprote n A II phenyto n

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Alpha or h gh-dens ty l poproteins (HDL) are decreased in many patients with coronary heart disease (3 25) and a low serum HDL cholesterol is associated w th an increased risk of develop ng ischem c heart d sease (11 21 22) These observations have raised the poss bil ty that any measure wh h increases the serum HDL concentrat on could retard the development of coronary heart d sease equally well or perhaps even better than hypolip dem c treatment

There are only a few factors wh ch are known to estrogenic hormones (?) ethanol (15 30) chlorinated hydrocarbon insecticides (6) and endurance exerc se (14 17 ?0 32) Minor increases are noted also during adm n stration of clofibrate (9 29 31) and in insul n treated diabet c patients (26) Of the above factors at least ethanol (28) and chlorinated hydrocarbons (8) are known to be liver m crosomal inducers We therefore thought that induct on of the hepatic microsomes might increase the synthesis of HDL Two of the most potent pharmacological agents known as m crosomal inducers viz phenobarb tone and phenyto n (8) have been shown to increase serum total cholesterol levels in man (23 27) To test the above hypothesis we determ ned serum l poprotein cholesterol and triglyceride and apol poprotein A levels in patients treated with phenytoin

PATIENTS AND METHODS

Twenty-one male and seven female pat ents with ep lepsy were stud ed All used phenyto n (diphenylhydanto n) as a regular ant convuls ve drug in doses vary ng from 200 to 300 mg da ly The pat ents were in good health and d d not use any other drugs Alcohol cs and persons known to use excess ve amounts of alcohol were excluded The phenyto n treatment had cont nued for 1 36 years The age of the pat ents was 46+16 years and the relat ve body weight was 112+21% (mean + S D)

Control subjects were healthy employees of an electric company part c pat ng n a health examination survey Lipoprote n data were ava lable from 44 males and 49 females with a mean age of 48+12 years and a relat ve body we ght of 113+23% Assays of apol poprote n A levels were carried out in a control group of 15 healthy males with a mean age of 47+7 years and mean relat ve body we ght of 106+9%

Venous blood was taken from pat ents and controls

Abb ev at ons HDL h gh-dens ty l poproteins LDL

Table I *Serum lipids and lipoproteins (mmoles/l mean ± S D) in phenytoin users and controls*

	Males		Females	
	Phenytoin users	Controls	Phenytoin users	Controls
Total cholesterol	6 8 ±1 4	6 8 ±1 3	6 0 ±1 5	6 8 ±1 4
Total triglyceride	1 79±0 89	1 48±0 75	1 05±0 69	1 08±0 53
VLDL cholesterol	0 74±0 55	0 67±0 49	0 34±0 28	0 44±0 26
VLDL triglyceride	1 19±0 79	0 94±0 55	0 52±0 48	0 49±0 31
LDL cholesterol	4 3 ±1 3	4 7 ±1 3	3 7 ±1 4*	4 8 ±1 4
LDL triglyceride	0 39±0 14	0 46±0 21	0 34±0 13	0 41±0 21
HDL cholesterol	1 70±0 50***	1 28±0 34	1 87±0 51***	1 52±0 31
HDL triglyceride	0 21±0 06	0 15±0 09	0 19±0 05	0 19±0 12

* $p<0.05$ *** $p<0.001$ for the difference phenytoin/control

after a 12 hour overnight fast No dietary instructions were given Serum was used for the assay of cholesterol (Boehringer kit) triglycerides (16) apolipoproteins A I and A II (1) and phenytoin (5) The major lipoproteins viz very low-density (VLDL) low-density (LDL) and high-density fractions were separated by flotation in a Sorvall preparative ultracentrifuge (13)

Plasma phenytoin levels of the patients ranged from 10 to 72 μmol/l (mean 34) The recommended therapeutic range is 40–80 μmol/l Thirteen patients (11 males and 2 females) had serum phenytoin above 40 μmol/l

The results are expressed as mean ± 1 S D The significance of the differences between patients and controls was calculated by Student s t test

RESULTS

The cholesterol and triglyceride concentrations in whole serum and in lipoproteins of phenytoin users and controls are given in Table I Both male and female patients treated with phenytoin had significantly higher HDL cholesterol levels than the respective controls Fig 1 shows the individual values of HDL cholesterol in the patients Twelve (43 %) patients had HDL cholesterol clearly above the upper limit of the normal range (mean + 2 S D) Increased concentrations were particularly common among the patients whose serum phenytoin level was within the recommended therapeutic range (above 40 μmol/l) Thus the mean HDL cholesterol in the male patients with adequate phenytoin dosage was 1 87±0 43 mmol/l against 1 51±0 57 mmol/l in males with suboptimal serum phenytoin levels

Serum total cholesterol and triglyceride concentrations of the phenytoin users did not differ significantly from those of controls The male patients however showed an unusually high prevalence of hypertriglyceridemia Thus 33 % of the phenytoin treated men had serum triglyceride above 2 0 mmol/l as compared to only 16 % in control men The phenytoin treated women on the other hand had a lower LDL cholesterol level than the respective control group

The apolipoprotein A I concentration was significantly increased in the male phenytoin users as compared to control men (Table II) Highest A values were again found in those patients whose serum phenytoin was within the therapeutic range The HDL cholesterol and apoprotein A I levels of phenytoin users showed a highly significant correlation with each other ($r=+0.77$ $p<0.001$) The molar ratio HDL cholesterol/apoprotein A I was simi

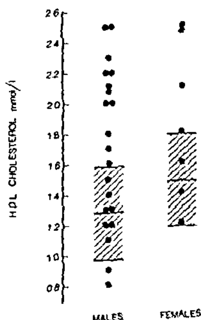


Fig 1 Serum HDL cholesterol values in epileptic patients on long term phenytoin medication ▨ = mean ± 1 S D of controls

ble II Serum apolipoprotein A I and A II concentrations (mg/100 ml mean±S D) in phenytoin treated and control males

	n	A I	A II
l phenytoin users	21	191±28 0**	51 0±17 7
rum phenytoin >40 μmol/l	11	205±28 4***	50 3±15 3
rum phenytoin <40 μmol/l	10	177±30 1*	51 6±20 7
ontrols	15	165±27 9	46 0±4 5

$p<0.05$ for the difference between the two phenytoin oups $p<0.01$ *** $p<0.001$ for the difference from ontrols

e in phenytoin treated (24 4) and control (24 3) nen No difference was found in the apoprotein A II levels of patients and controls

DISCUSSION

The present study was based on a working hypothesis that serum HDL concentration could be increased by agents which cause hypertrophy of the liver microsomes The possibility that this could be the case was raised by previous observations on the occurrence of hyper-α lipoproteinemia in alcoholics (15 24 30) in moderate drinkers (4 7) and in persons exposed to chlorinated hydrocarbon insecticides (6) Both ethanol (28) and chlorinated hydrocarbons (8) are known to increase the amount of smooth endoplasmic reticulum in the liver Furthermore two other microsomal inducers viz phenobarbitone and phenytoin had previously been shown to increase the serum cholesterol level (23 27) and it seemed possible that this rise could be accounted for by HDL cholesterol The results f the present study support our hypothesis but do ot prove it because we lack final evidence first nat the increase of HDL detected in our patients as related to phenytoin use and second that the atients had a hypertrophy of hepatic microsomes Ultimately the site and mechanism of the hepatic synthesis of nascent HDL are not yet known The smooth endoplasmic reticulum which is the main subcellular structure stimulated by drugs is probably the site in liver cells where the lipoprotein lipids are synthesized and organized to particles (10) In view of this it is interesting that also the serum triglyceride and VLDL levels tended to be higher in our male phenytoin users than in respective controls An increase in serum VLDL has been previously observed after administration of phenobarbitone in man (23) and of a chlorinated hydrocarbon in rat (12)

Since serum HDL shows an inverse correlation with the risk of coronary heart disease (3 11 21 22 25) one might expect that epileptic patients using phenytoin through years are protected from this disease To our knowledge there are no systematic studies in support of this possibility but two independent reports have recently mentioned that many clinicians taking care of epileptic patients have been impressed by the low incidence of myocardial infarction among them (18 19) This observation if confirmed fits our present data and serves as an example of a possibly beneficial side-effect of a drug

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Acquired von Willebrand's Disease Caused by a Monoclonal Antibody

Olle Zettervall and Inga Marie Nilsson

From the Department of Internal Medicine and the Coagulation Laboratory University of Lund Malmo General Hospital Malmo Sweden

STRACT A 67 year old man with malignant phoma and acquired von Willebrand s disease is cribed His bleeding symptoms started late in life d at this time a monoclonal IgGK serum protein is found He had a prolonged bleeding time de eased platelet adhesiveness, low values for factor II clotting activity (VIII C) factor VIII related tigen (VIIIR Ag) and ristocetin co-factor activity III Rcof) Infusion of factor VIII concentrates raction I–0) did not correct the abnormalities No hibitory activity in vitro of the patient's plasma or G fraction could be demonstrated against VIII C, IIIR Ag and VIII Rcof In order to demonstrate n antibody that binds factor VIII without inhibiting s biological activities in vitro, advantage was taken the fact that staphylococcal protein A strongly nds the Fc part of IgG molecules Addition of aphylococci to mixtures of patient IgG and a factor III preparation resulted in removal of all factor III activities (VIII C, VIIIR Ag, VIII Rcof) from e supernatant at sedimentation of the bacteria The ctive binding principle was the M-component, i e robably a monospecific antibody molecule We ypothesize that the complex is formed in vivo and liminated at an accelerated rate

Key words von Willebrand's disease lymphoma mono-clonal immunoglobulin staphylococcal protein A

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von Willebrand's disease is an inherited haemorrhagic disorder with a long bleeding time low factor VIII coagulant activity and decreased platelet adhesiveness (21 30) Patients with von Willebrand s disease also show a typical response to infusion of factor VIII concentrates with a retarded increase in factor VIII activity (20) In recent years some other usual features of von Willebrand s disease have been revealed namely a low level of factor VIII related antigen (38) and an impaired or absent platelet aggregation in plasma by the antibiotic ristocetin (9) Application of these techniques has disclosed that von Willebrand s disease is heterogeneous and several variants have been described (6 8 13 27 35)

Acquired disorders which resemble von Willebrand s disease have also been reported As far as we know 13 such cases are on record and most of these patients had immunologic or lymphoproliferative disorders (10 11 14 17 18 28 32 33 34) (Table I) It appears that in all reported cases except those of Handin et al (4) and Wautier et al (37) no inhibitor has been detected in plasma and the mechanism responsible for the defective function of the factor VIII protein complex in acquired von Willebrand s disease is largely unknown

This report describes a patient with malignant lymphoma and acquired von Willebrand s disease His serum contained a monoclonal protein of IgGK class No direct inhibitory activities were demonstrable in plasma but it was possible to show that the purified IgG complexed with the factor VIII protein molecule in vitro explaining the rapid turnover of all activities combined with the factor VIII protein complex in vivo

METHODS AND MATERIALS

Platelet count Ivy bleeding time activated partial thromboplastin time (APTT) one stage prothrombin

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Requests for reprints Professor I M Nilsson Coagulation Laboratory Allmanna Sjukhuset 21401 Malmö Sweden

Abbreviations VIII C=factor VIII clotting activity VIIIR Ag=factor VIII related antigen VIII Rcof= ristocetin co-factor AP[illegible] activated partial thromboplastin [illegible]lasma

Table I *Cases of acquired von Willebrand (vW) syndrome*

Author	Underlying disease	VIII C (%)	VIIIR Ag (%)	BT (min)	VIII Rcof (%)	Antibody	Transfusion studies
Simone et al (1968)	SLE	4–17	–	10	–	Neg	–
Veltkamp et al (1970)	Pesticide ingest	3	–	15	–	Neg	
Ingram et al (1971 1973)	1) M-comp	20–35	40	14	–	Neg	No vW respon
	2) SLE	2–22	–	14	–	Neg	Rapid decrease of VIII C
	3) None	7–20	5	20	–	Neg	–
	4) None	4–18	5	20	–	Neg	Rapid decrease of VIII C
Pool & Jones (1972)	SLE	15	20				
Mant et al (1973)	M-comp	4–20	4	Prolonged	–	Neg	No vW respons
Leone et al (1974)	SLE	15	20	Prolonged	–	Neg	No vW respons
Meyer et al (1974)	M-comp	10	5	Prolonged	–	Neg	No vW respons
Stableforth et al (1975)				15	Absent	–	–
	Diabetes	1 5	10	20	Absent	VIII C	No vW respons
Wautier et al (1976)	CLL	52	10–20	20	6–10	VIII Rcof (platelet eluate)	No vW respons
Handin et al (1976)	Lymphosarc M-comp	14–25	10–20	20	Decreased	IgA VIII Rcof IgG	No vW response
Present case	Lymphoma M comp	36	25	15	26	VIII C VIIIR Ag VIII Rcof IgG	No vW response

time prothrombin + factor VII + factor X (Owren s P&P test) factor V factor IX and fibrinogen were determined according to methods described earlier (22 25) Platelet adhesiveness was performed according to the methods of Hellem (5) and Salzman (30) VIII C was assessed from its normalizing effect on the recalcification time of platelet rich haemophilia A plasma containing less than 1% of VIII C (22) VIIIR Ag was determined with Laurell s electroimmunoassay (6) VIII Rcf was determined as described by Macfarlane et al (15)

Test for inhibition of VIII C VIIIR Ag and VIII Rcof The inhibiting effect of the patient s plasma and IgG fraction was determined by incubating 1 part of the patient s plasma or 1 part of the IgG fraction (1 mg/ml) with 4 parts of normal plasma for 1 hour at 37°C As a blank 1 part of plasma from a patient with severe von Willebrand s disease or 1 part of 5% albumin solution or 1 part of normal IgG (gammaglobulin Kabi) (1 mg/ml) were incubated with the normal plasma After incubation the mixtures were assayed for the residual VIII C VIIIR Ag and VIII Rcof

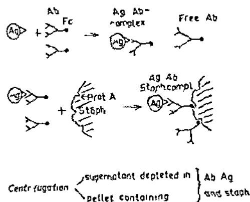
Two-stage method for assay of inhibitor against factor VIII This test was performed as described by Nilsson and Hedner (23)

Crossed immunoelectrophoresis in agarose was done according to Ganrot (3)

Platelet eluates were prepared from platelet rich plasma (PRP) of normal plasma and patient plasma as described by Stableforth et al (33)

Preparation of IgG from patient plasma Citrated plasma was made half saturated in ammonium sulphate and the mixture was allowed to stand for 1 hour The resulting precipitate was collected dialysed exhaustively against 0 0175 M pH 6 5 phosphate buffer and the crude globulin solution then chromatographed on a DEAE Sephadex A 50 column equilibrated and eluted with the same buffer On agarose electrophoresis the first portions of the eluate (fractions 20–45) contained predominantly M component whereas late fractions were rich in polyclonal IgG

Separation of IgG antibody–antigen complexes from reaction mixtures In experiments designed to demonstrate binding between IgG antibody and factor VIII immune complexes (and free antibody) were removed from the antigen–antibody mixtures with a protein A immunosorbent This was either heat and formalin stabilized washed Cowan A staphylococci or protein A Sepharose (Pharmacia Uppsala Sweden) When bacteria were used 1 volume of reaction mixture received 1 volume of 10% (v/v packed staphylococci) bacterial suspension in 0 075 M pH 7 4 tris buffer After brief mixing bacteria were spun down and the supernatant was assayed for residual VIII C VIIIR Ag and VIII Rcof and sometimes the pelleted bacteria were recovered washed and analysed for factor VIII C (see below) Using protein A Sepharose aliquots of a suspension in tris buffer of washed swelled immunosorbent were pipetted into test tubes After centrifugation the supernatants were carefully sucked off Reaction mixtures were immediately pipetted on the gel pellets and subsequently the experimental procedure was carried out as with the bacterial immunosorbent The amount of IgG in the reaction mix



l Sedimentation of IgG antibody (*Ab*)-antigen (*Ag*) nplexes with staphylococcal protein A immunosorbent tein A binds to the Fc part of the antibody

es was always kept below the binding capacity of phylococci or protein A Sepharose (Fig 1)

Protein determination Solutions of IgG were read at) nm and an $A^{1\%}_{280}$ of 13 6 was assumed

Immunochemical determination of IgG Single radial munodiffusion (16) was used with either specific rabbit tiserum against Fc fragments of normal human IgG (gift om Dr A Grubb) or anti lambda chain serum (Dakopats S Copenhagen Denmark) The method was applied to pernatants in immunosorbent and preparative electrophoresis experiments in which only relative concentration lues were of interest Therefore dilutions of the IgG ed were employed for calibration

Preparative electrophoresis Twenty µl of patient IgG iostly M-component) of concentration 35 mg/ml was ectrophoresed in a standard agarose plate for analytical ectrophoresis (12) Equal gel fractions (1×2×20 mm) ere cut with razor blades using a simple spacer The gel rips were soaked in 200 µl of phosphate buffered saline the cold for 72 hours The eluates were screened for IgG ontent and factor VIII binding activity with simple radial nmunodiffusion and the staphylococcal immunosorbent chnique respectively

Determination of VIII C activity in staphylococcal pellets Pellets (0 1 ml) were washed twice in 1 ml of tris uffer (0 075 M pH 7 4) The bacteria were then uspended in 0 7 ml of haemophilia A test plasma The uspensions were recalcified by addition of 0 8 ml of $CaCl_2$ olution (30 mM) and coagulation times noted VIII C ctivity in the washings was monitored with a similar rocedure using 0 1 ml of washing buffer instead of taphylococcal pellet

Factor VIII concentrate Human fraction I-0 prepared by AB Kabi (AHF Kabi) according to the glycine method of Blomback and Blomback (2) One bottle (100 ml) contains about 300 U of VIII C The characteristics of this preparation have been studied (24) The activity of the concentrate expressed in U/ml solution (1 U = the amount of activity present/ml standard plasma) is for VIII C about 3 U/ml for VIIIR Ag about 6 U/ml and for VIII Rcof 5 U/ml

CASE HISTORY

The patient is a 67 year-old foreman who belongs to a sibship of 8 children With the exception of the patient s mother, who had been troubled by nose bleedings when old no cases with an increased bleeding tendency were known in his family The patient has two sons On investigation they had normal bleeding time and normal values for VIII C VIIIR Ag and VIII Rcof The patient s earlier medical history is inconspicuous with the exception of vague abdominal symptoms probably attributable to cholecystopathy and tendency to nose bleedings already apparent in his youth Otherwise he had noted no bleedings from mucous membranes and no easy bruisability At the age of about 20 he underwent extensive tooth extractions without excessive bleedings and blood transfusions were not required

In 1974 the patient had an episode of severe epistaxis after a few days ingestion of aspirin for a headache Local pressure had also to be applied to stop bleedings from the sites of venipuncture A prolonged bleeding time was noted The patient was referred from his local hospital for a coagulation study at our laboratory The coagulation analyses (March 1974 when the patient had been off aspirin for 3 weeks) (Table II) showed abnormalities compatible with a diagnosis of von Willebrand s disease At his local hospital it had on this occasion been found that the plasma protein electrophoresis showed a protein (M component) of immunochemical class IgGλ (concentration 6 g/l)

The patient was well until Aug 1976 when he noticed epigastric pain He was again admitted to his local hospital There had been no gross bleeding but he was severely anaemic with a Hb of 39 g/l and had positive Weber tests for occult fecal blood The effect of blood transfusions on the Hb level was only temporary and the patient was referred to the Surgical Clinic in Malmo for further investigation At endoscopy an ulcerated gastric tumour was diagnosed He received infusions of AHF Kabi (Fig 2) and on Nov 25th gastric resection according to Billroth II was made together with cholecystectomy (cholecystopathy had been known for several years) The

Table II Coagulation studies

	March 1974	Nov 11 1976	Normal
Platelet count (10^9/l)	179	220	125–340
Bleeding time (min)	15	15	6–12
Platelet adhesiveness			
Salzman (%)	12	12	20–60
Hellem (%)	24		17–33
APTT (sec)	90	120	29–41
VIII C (%)	30	36	60–160
VIIIR Ag (%)	28	25	50–160
VIII Rcof (%)		26	50–160
F IX (%)		90	60–160
P&P (%)	77	93	
FV (%)	95	174	
Fibrinogen (g/l)	3.3	4 1	
Antibody to VIII C (U/ml)			

Table I *Cases of acquired von Willebrand (vW) syndrome*

Author	Underlying disease	VIII C (%)	VIIIR Ag (%)	BT (min)	VIII Rcof (%)	Antibody	Transfusion studies
Simone et al (1968)	SLE	4–17	–	10	–	Neg	–
Veltkamp et al (1970)	Pesticide ingest	3	–	15	–	Neg	
Ingram et al (1971 1973)	1) M-comp	20–35	40	14	–	Neg	No vW response
	2) SLE	2–22	–	14	–	Neg	Rapid decrease of VIII C
	3) None	7–20	5	20	–	Neg	–
	4) None	4–18	5	20	–	Neg	Rapid decrease of VIII C
Pool & Jones (1972)	SLE	15	20	Prolonged	–	Neg	No vW response
Mant et al (1973)	M-comp	4–20	4	Prolonged	–	Neg	No vW response
Leone et al (1974)	SLE	15	20	Prolonged	–	Neg	No vW response
Meyer et al (1974)	M-comp	10	5	15	Absent	–	–
Stableforth et al (1975)	Diabetes	1 5	10	20	Absent	VIII C	No vW response
Wautier et al (1976)	CLL	52	10–20	20	6–10	VIII Rcof (platelet eluate) IgA	No vW response
Handin et al (1976)	Lymphosarc M comp	14–25	10–20	20	Decreased	VIII Rcof IgG	No vW response
Present case	Lymphoma M-comp	36	25	15	26	VIII C VIIIR Ag VIII Rcof IgG	No vW response

time prothrombin + factor VII + factor X (Owren s P&P test) factor V factor IX and fibrinogen were determined according to methods described earlier (22 25) Platelet adhesiveness was performed according to the methods of Hellem (5) and Salzman (30) VIII C was assessed from its normalizing effect on the recalcification time of platelet rich haemophilia A plasma containing less than 1% of VIII C (22) VIIIR Ag was determined with Laurell s electroimmunoassay (6) VIII Rcf was determined as described by Macfarlane et al (15)

Test for inhibition of VIII C VIIIR Ag and VIII Rcof The inhibiting effect of the patient s plasma and IgG fraction was determined by incubating 1 part of the patient s plasma or 1 part of the IgG fraction (1 mg/ml) with 4 parts of normal plasma for 1 hour at 37 C As a blank 1 part of plasma from a patient with severe von Willebrand s disease or 1 part of 5% albumin solution or 1 part of normal IgG (gammaglobulin Kabi) (1 mg/ml) were incubated with the normal plasma After incubation the mixtures were assayed for the residual VIII C VIIIR Ag and VIII Rcof

Two-stage method for assay of inhibitor against factor VIII This test was performed as described by Nilsson and Hedner (23)

Crossed immunoelectrophoresis in agarose was done according to Ganrot (3)

Platelet eluates were prepared from platelet rich plasma (PRP) of normal plasma and patient plasma as described by Stableforth et al (33)

Preparation of IgG from patient plasma Citrated plasma was made half saturated in ammonium sulphate and the mixture was allowed to stand for 1 hour The resulting precipitate was collected dialysed exhaustively against 0 0175 M pH 6 5 phosphate buffer and the crude globulin solution then chromatographed on a DEAE Sephadex A 50 column equilibrated and eluted with the same buffer On agarose electrophoresis the first portions of the eluate (fractions 20–45) contained predominantly M-component whereas late fractions were rich in polyclonal IgG

Separation of IgG antibody-antigen complexes from reaction mixtures In experiments designed to demonstrate binding between IgG antibody and factor VIII immune complexes (and free antibody) were removed from the antigen–antibody mixtures with a protein A immunosorbent This was either heat and formalin stabilized washed Cowan A staphylococci or protein A Sepharose (Pharmacia Uppsala Sweden) When bacteria were used 1 volume of reaction mixture received 1 volume of 10% (v/v packed staphylococci) bacterial suspension in 0 075 M pH 7 4 tris buffer After brief mixing bacteria were spun down and the supernatant was assayed for residual VIII C VIIIR Ag and VIII Rcof and sometimes the pelleted bacteria were recovered washed and analysed for factor VIII C (see below) Using protein A Sepharose aliquots of a suspension in tris buffer of washed swelled immunosorbent were pipetted into test tubes After centrifugation the supernatants were carefully sucked off Reaction mixtures were immediately pipetted on the gel pellets and subsequently the experimental procedure was carried out as with the bacterial immunosorbent The amount of IgG in the reaction mix

able III *Tests for inhibition of VIII C VIIIR Ag d VIII Rcof (%)*

o 4 parts of normal asma was added part of	VIII C	VIIIR Ag	VIII Rcof
lbumin 5 %	58	65	58
asma from the atient	65	72	68
asma from a patient ith severe vW	68	61	46
ammaglobulin Kabi mg/ml	68	91	84
G from the patient mg/ml	51	76	58

Table IV *Staphylococcal protein A binding experiments in mixtures of factor VIII and IgG (patient and normal)*
Assays of the supernatant after centrifugation

System	VIII C (%)	VIIIR Ag (%)	VIII Rcof (%)
1 vol F VIII + 1 vol patient IgG + 2 vol staph 10%	10	39	23
1 vol F VIII + 1 vol normal IgG + 2 vol staph 10%	24	92	62
Values calculated according to the dilution	25	75	68

No inhibitor against VIII C could be detected in e patient s plasma using the two-stage assay for ctor VIII inhibitor From Table III it is also ap arent that addition of the patient s plasma to nor al plasma did not cause any significant decrease in III C VIIIR Ag or VIII Rcof

Platelet eluates were prepared from PRP of nor al plasma and patient s plasma as described by tableforth et al (33) The effect of the eluates n VIII Rcof was tested by adding 0 5 and 0 2 l respectively to control PRP (total vol /ml) No hibition of VIII Rcof was observed

As mentioned the patient had a monoclonal prote in of immunochemical class IgGK at a concentra ion of 6 g/l Four weeks after the operation a total f 5 double plasmaphereses were performed using enwal bags during the course of one week The oncentration of the IgGK component did not de rease and the levels of VIII C VIIIR Ag and III Rcof remained unchanged

Since no inhibitory activities could be demon trated in the patient s plasma his plasma IgG com onent was prepared and tested for any inhibitory ction On addition of this component to normal lasma (Table III) however no inhibitory effect as observed

In order to demonstrate the presence of factor III-anti factor VIII complexes in mixtures of pa ient IgG and factor VIII advantage was taken of he fact that staphylococcal protein A (present on he surface of Cowan A staphylococci) strongly inds to the Fc part of IgG molecules Thus the ddition of staphylococci to such mixtures would esult in binding of immune complexes (and free antibody) to the bacteria and removal of bound factor VIII from the supernatant at sedimentation of the bacteria (see Methods) As illustrated in Table IV partial depletion of supernatant VIII C VIIIR Ag and VIII Rcof did in fact occur That these activities were brought down with the bacteria (and not merely inactivated) is shown in Table V Patient IgG clearly performed better in binding factor VIII to the staphylococci than did normal IgG or buffer When checked supernatants contained less than 10% of the original amount of IgG a result substantiating the efficacy of the bacterial immunosorbent Depletion of factor VIII activities was also achieved when purified and insolubilized protein A (protein A Sepharose) was substituted for bacteria Experiments with patient plasma gave similar results

When patient IgG was subjected to preparative electrophoresis and the fractions were assayed for factor VIIIR Ag binding activity this could be correlated to the presence of M component in the samples and not to that of light chain class lambda representing polyclonal IgG (Fig 3) Confirmatory

Table V *VIII C in staphylococcus pellets*

Incubation mixture Experiment no	Recalcification time (sec) 1	2	3
Buffer	360	n d	n d
Patient IgG (1 mg/ml) + F VIII	70	89	99
Normal IgG (1 mg/ml) + F VIII	129	103	125

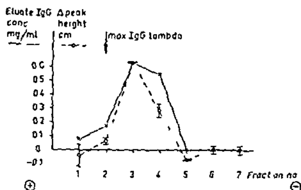


Fig 3 Preparative agarose electrophoresis of plasma IgG from the patient Equal gel fractions were cut and eluted in standard volumes of buffer Eluates were tested for content of IgG Ig of light chain type lambda and VIIIR Ag binding capacity (given as Δ peak height at Laurell rocket electrophoresis of staphylococcus supernatants) Max IgG lambda is supposed to indicate the bulk of polyclonal IgG

results were obtained when IgG fractions from DEAE chromatography of patient serum were used instead of the electrophoretic preparations briefly at the same protein concentration M component rich IgG bound factor VIII better than protein with predominantly polyclonal IgG

DISCUSSION

This 67 year old man with a malignant lymphoma had an acquired von Willebrand syndrome induced by an IgGK antibody interacting with the factor VIII protein complex He had no family history of bleeding and his bleeding symptoms started late in life in connection with the detection of the myeloma protein (M component) in his serum His coagulation analysis was typical for that seen in classical von Willebrand s disease with a prolonged bleeding time decreased platelet retention low values for VIII C VIIIR Ag and VIII Rcof It has been repeatedly shown that fraction I-0 (AHF Kabi) corrects the abnormalities in von Willebrand s disease and produces a retarded rise of VIII C (7 20 31) In this patient there was both a poor recovery and a rapid disappearance of VIII C The half life of VIIIR Ag is reported to be about 24 hours (1 7) and that of VIII Rcof about 12–20 h (24) In our patient these activities disappeared much more rapidly and the recovery was also poor These findings indicated either the presence of an inhibitor in the patient s plasma or that the factor VIII protein complex was metabolized much more quickly tha normal Antibody activity of monoclonal immunoglobulins is well recognized (29) and seemed likely cause of the development of an acquired von Willebrand s syndrome in this patient However when the patient s plasma or the IgGK fraction of his serum was added to normal plasma or fraction I-0 no inhibition of VIII C VIIIR Ag or VIII Rcof could be demonstrated

The finding that factor VIII activities could be removed from a factor VIII enriched preparation when patient IgG had been added prior to absorption with staphylococci but hardly so when normal IgG or a buffer blank had been used strongly indicates that patient IgG specifically reacts in some way with factor VIII Considering that factor VIII co sedimented with the staphylococci and that protein A on staphylococcal surfaces binds IgG it seems likely that patient IgG acted as a multivalent ligand connecting factor VIII and bacteria Since antibody complexed antigens are known to be eliminated more rapidly in vivo than free antigen (26 36), the low values of VIII C VIIIR Ag and VIII Rcof in the patient s plasma as well as the rapid disappearance of these activities after infusion of factor VIII concentrate could also be explained by complex formation The active binding principle was evidently the M component

Of the 14 reported cases of acquired von Willebrand s syndrome at least 11 had some abnormality in the immunologic system (Table I) Thus 5 subjects (4 10 17 18) had an IgG monoclonal gammopathy 4 subjects had SLE (10 14 28 32) one patient had chronic lymphocytic leukaemia (37) and one a reaction to a pesticide that was probably a hypersensitivity reaction (34) In the 4 patients with SLE (11 14 28 32) the coagulation abnormalities were corrected by steroid therapy As mentioned in the case of Handin et al (4) an inhibitor which prevented aggregation of normal platelets by ristocetin was present in the IgG fraction of the patient s serum and in the case of Wautier et al (37) in the IgA fraction In none of the other cases could any inhibitory activity against factor VIII activities be demonstrated in the plasma Infusion studies were done in the cases described by Mant et al (17) Wautier et al (37) Veltkamp et al (34) Handin et al (4) and Leone et al (14) In all these cases it was apparent that there was no secondary rise in VIII C and that the effect on bleeding time and platelet adhesiveness rapidly disappeared

It seems highly probable that an immune complex ormation between patient IgG and factor VIII simi ir to that demonstrated in our case was present in ie other reported cases Newman et al (19) have :cently questioned the existence of factor VIII in lasma as a complex of exceedingly high molecular e ght The d stribution of VIIIR Ag and VIII C n ultrafiltration across membranes (Amicon filters) ius suggested that factor VIII exists in the circulat g blood as two substances of relatively low iolecular weight 200000 (VIII C) and 240000 VIIIR Ag and VIII Rcof) However the observa ons in our patient with a supposedly monospecific it body and a concomitant decrease in all factor III act vities spontaneously as well as after factor III infusions ind cate that VIII C VIIIR Ag and III Rcof are indeed connected in a molecular omplex n vivo Such a complex should exist in qu l brium with its subunits But in vivo the bulk f the activit es are obviously connected with the omplex The finding of ultraf lterable su b units oes not exclude the existence of the complex

The affinity of the patient s M component f r t tor VIII is evidently low as high molar excess c f ie antibody is required for b nding Clinic illy ven a modest decrease of the M component may ierefore result in considerable improvement c f the oagulation and bleeding disorder

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